

CANADIAN JOURNAL OF ANIMAL SCIENCE

The official publication of the Canadian Society of Animal Production

VOLUME 38

DECEMBER, 1958

No. 2

RAISING DAIRY CALVES WITHOUT WHOLE MILK

J. M. BELL¹

Department of Animal Husbandry, University of Saskatchewan, Saskatoon, Saskatchewan

[Received for publication July 25, 1957]

ABSTRACT

The results of feeding experiments, involving a total of 117 Holstein-Friesian calves, were reported. No whole milk nor fresh skimmilk was fed to the calves after their second day of age. Milk substitutes containing 40 to 50 per cent dried skimmilk, 5 to 10 per cent emulsified, stabilized lard and at least 30 p.p.m. of Aureomycin (chlortetracycline) or Terramycin (oxytetracycline) in the dry matter permitted weight gains equal to, or exceeding, the Beltsville standards during the milk feeding period. Supplementary lecithin had no effect on performance of the calves. Weaning calves from milk substitutes at weights of 145 to 150 lb. was considered preferable to weaning at specified ages because of apparent differences in physiological development that exist among calves for the first few months of life.

INTRODUCTION

Methods for raising dairy calves successfully without extensive feeding of whole milk have been under study for many years. Among recent developments, Norton and Eaton (7) reported above-normal growth for calves fed about 350 lb. of whole milk together with suitable dry calf starters. Whiting and Clark (11) obtained good results with calves fed about 235 lb. of whole milk up to the fourth week of age and followed by either skimmilk feeding or a dry starter ration. Following studies of milk substitutes, Wallace *et al.* (10) found that calves weaned from whole milk when 10 to 14 days of age grew slightly slower than normal for a few weeks and appeared rough-coated but recovered satisfactorily by the time they reached the age of 4 to 6 months. Hopper *et al.* (2) fed skimmilk and re-constituted skimmilk containing butyrated lard to veal calves from 3 days of age and reported excellent growth to 56 days of age.

It is evident that most studies have involved the use of appreciable quantities of salable whole milk. Attempts to reduce the feeding of whole milk frequently led to scours. Since such digestive disorders in calves usually involve intestinal micro-organisms, the advent of antibiotics for feed use offered a new approach to these problems. Several recent reports testify to the value of antibiotics in this regard (3,4,5,9).

This report concerns experiments with Holstein-Friesian calves fed various milk substitutes, in place of whole milk, beginning on the third day after birth.

¹ Professor and Head of Department of Animal Husbandry, University of Saskatchewan, Saskatoon, Sask.

MATERIALS AND METHODS

The calves used in these studies were produced in the University herd during the fall and early winter and were left with their dams for 2 days following birth. After this they were housed in individual pens in a clean, warm barn and were allowed access to fresh water, starter rations and hay. Milk substitutes were fed twice daily, except to certain of the smaller calves in which cases feeding was done three times daily for several days.

Preliminary trials were conducted during the winters of 1952-53 and 1953-54. In the first year, 33 calves were used in a comparison of two formulas, one based largely on milk by-products and the other employing cereal grain by-products as a partial substitute for milk powders. The formulas were: (a) skim milk powder 40, buttermilk powder 43, lard (stabilized 10), oxytetracycline (Terramycin¹) 0.8, brewers' dried yeast 3 per cent, plus trace minerals and synthetic A & D vitamin preparations; (b) buttermilk powder 60, soybean flour 10, oat flour 12, wheat flour 6.5, brewers' dried yeast 3, vitagrass 5, bone meal 1.25, rock phosphate 0.6, salt 0.5, soybean oil 0.25, feeding fish oil (1500 A, 300 D/gm.) 0.2 per cent, plus trace minerals.

In this test, calves fed formula (a) grew well, appeared very thrifty and displayed no signs of digestive disturbance at any age. Much variability was obtained with the second formula and diarrhoea was common. Addition of Aureomycin² and sulphathalidine to the ration frequently failed to effect a cure.

In the second year, 23 calves were fed rations based on the best formula used previously. The amount of Terramycin was reduced, however, to 10 p.p.m. (dry basis) from the 50 p.p.m. used before. This test provided evidence of instability of the vitamin A employed and some indication that 10 p.p.m. of antibiotic was too low to control scours. Having obtained a formula that would permit normal growth (Beltsville standard, 6) without employing fresh whole milk, the following experiments were designed:

Experiment 1 (1954-5)

A new formula containing 30 p.p.m. Aureomycin, stabilized vitamin A and additional riboflavin was developed (Table 1). The experimental design involved four groups in a 2 x 2 factorial arrangement: two levels of energy (T.D.N.) each with or without supplementary lecithin. The calves were allotted in rotation, within sex groups, in order of date of birth to the various test lots.

One-half of the calves received a daily maximum milk replacer allowance that supplied the T.D.N. requirement (8) of calves weighing 100 lb. The other calves were allowed 25 per cent more T.D.N. Both lots were held to their respective milk replacer levels throughout the fluid feeding period of 6 to 8 weeks. Individual records of starter and hay consumption up to 56 days of age were kept.

One-half of the calves receiving each level of energy from milk replacer were fed supplemental lecithin³ to provide an amount equal to 2 per cent of

¹ Bi Con 3 plus 3. (Chas. Pfizer & Co., Inc., Chicago, Ill.).

² Aureomycin T.-F. (Lederle Laboratories Division, Pearl River, N.Y.).

³ Sta-Sol lecithin concentrate (vegetable). (A. E. Staley Mfg. Co., Decatur, Ill.). 65% soybean phosphatides in 35% soybean oil.

TABLE I—COMPOSITION OF MILK REPLACER FORMULA

Ingredients	Amount per 100 lb.	
Skimmilk powder (spray dried)	50	lb.
Whey powder	20	lb.
Oat flour	19	lb.
Lard, emulsified, stabilized	5	lb.
Brewers' dried yeast	5	lb.
Aurofac D ¹	0.3	lb.
Salt, cobalt-iodized	0.5	lb.
Vitamin A supplement ²	50	gm.
Vitamin D ₂ supplement ³	60	gm.
Riboflavin supplement ⁴	2.5	gm.
Iron sulphate	5	gm.

¹ Contained 5 gm. chlortetracycline per lb.

² Myva Dri A, 20,000 I.U. per gram.

³ Synthetic vitamin D₂, 150,000 I.U. per lb.

⁴ Merck's Riboflavin No. 54, 16 gm. per lb.

the milk replacer dry matter. The value of lecithin was in doubt because, while several milk replacer formulas have included lecithin, the information available on its value for calves is limited. Esh *et al.* (1) reported that colostrum-free calves required lecithin for survival.

Experiment 2 (1955-6)

This investigation was designed to obtain data on 1) the effects of 'weaning' calves according to weight rather than age; 2) the effects of feeding hay and concentrates mixed together versus feeding separately, and 3) feed consumption of calves up to 56 days of age.

Lot I calves, starting at 3 weeks of age, were offered a mixture of two parts starter formula to one part chopped alfalfa hay. Lot II calves were allowed similar feeds in separate boxes. Free access was allowed to the starter ration for all calves until 3 weeks old. When the calves reached 56 days of age a grower ration replaced the starter ration and was allowed up to a maximum of 5 lb. per calf daily. Alfalfa hay was fed *ad libitum* and a small allowance of good quality grass silage was given.

Weights of calves were taken every 2 weeks and when 145 lb. was attained weaning was commenced. This was done by gradually reducing the liquid allowance over a period of 4 or 5 days. After 'weaning', live-weights were taken monthly.

The concentrate formulas were as follows: *Starter*: ground barley 40, ground oats 18, linseed oilmeal 15, soybean oilmeal 15, bran 10, bone meal 1, cobalt-iodized salt 1 (all in pounds), and Aurofac 2A¹ 34 gm. *Grower*: ground wheat 25, ground oats 47, linseed oilmeal 15, bran 10, bone meal 2 and cobalt-iodized salt 1 lb.

Analysis of Growth Data

The Beltsville growth standards for Holstein cattle (6) were used for comparison in these studies and, for additional simplification, the weights

¹ Aurofac 2A contained 3.6 gm. chlortetracycline per lb. (Lederle Laboratories Division, Pearl River, N.Y.).

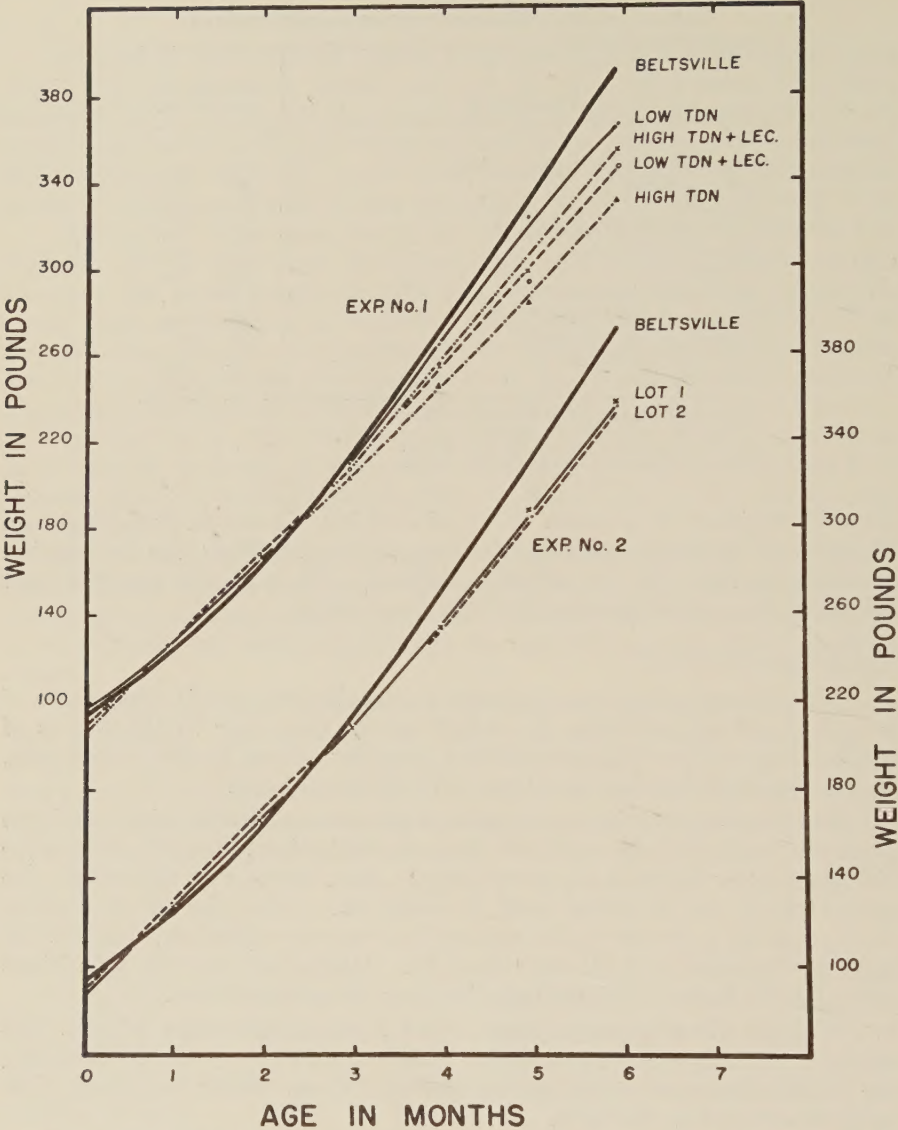


FIGURE 1. Growth curves of calves in Experiments No. 1 and No. 2 shown in relation to the Beltsville Growth Standards for heifers.

for each calf were expressed as percentages of the Beltsville standards for calves of the same weight and sex.

RESULTS AND DISCUSSION

Experiment 1

The results of this study of milk replacer intake levels and of supplementary lecithin are shown in Figure 1. No differences were apparent between any of the four treatments during the liquid feeding period, nor

until the calves were about 3 to 4 months of age. After this time, the responses to the low intake level without added lecithin did attain statistical superiority ($P = < .05$) over the same intake level with lecithin. The significance of this finding was not apparent from this study.

Analysis of variance of the growth data revealed, as a general effect, a significant increase in rate of gain as compared to the Beltsville standards, at 30 and 60 days of age; gains equal to the standards at 90 and 120 days but falling significantly below by 180 days of age. In view of more recent experience, and other investigations (11), it is possible that the restriction of concentrates to 3 lb. daily per calf after the calves were 90 days old was responsible for the decreased growth rates between the third and sixth month.

This experiment indicated no advantage in feeding more than 2 lb. of milk replacer dry matter daily per calf and no advantage in adding lecithin. The modified replacer formulas containing 30 p.p.m. Aureomycin, along with increased amounts of vitamins A, D and riboflavin, apparently eliminated the problems of scours, swollen joints and impaired vision such as occurred in the preliminary studies.

It was often observed that calves which were much smaller than average at birth appeared to be too immature, physiologically, to be 'weaned' at 6 weeks of age without danger of digestive disorders.

Experiment 2

The feed consumption and growth data for the calves, until they were 8 weeks of age, are shown in Table 2 and the growth curves to 6 months of age are presented in Figure 1. There was no obvious advantage in either method of feeding starter and hay as far as gains were concerned although several calves displayed tendencies to sort the mixtures and to consume more concentrates than roughages.

TABLE 2.—GROWTH AND FEED CONSUMPTION RESULTS FROM CALVES FED HAY AND STARTER TOGETHER VERSUS SEPARATELY

	Hay and starter together		Hay and starter separately	
	Males	Females	Males	Females
Number of calves	7	8	9	6
Average growth				
Initial weight, lb.	89	87	90	82
Weight at 56 days, lb.	169	166	175	165
Age at 150 lb., days	45	46	40	48
Daily gain to 150 lb., lb.	1.36	1.37	1.48	1.44
Beltsville standard weight, 60 days, lb.	171	160	171	160
Average feed consumption				
Starter ration eaten to 3 weeks, lb.	4.0	4.5	6.1	5.3
Starter ration eaten, 3 to 8 weeks, lb.	—	—	78	57
Starter-hay mixture, 3 to 8 weeks, lb.	65	75	—	—
Hay eaten, 3 to 8 weeks, lb.	—	—	13	17
Milk replacer fed, dry basis, lb.	81	70	80	89

The data indicate that the calves in this experiment equalled or surpassed the Beltsville standards at 30, 60 and 90 days of age, even though they were only 90 per cent of the 'standard' weight at birth. Thereafter, the rates of growth decreased slightly to 91 per cent of the standard weight at 6 months of age. It is possible that the initial advantages in weight-for-age might have been maintained if more than 5 lb. of concentrate had been allowed daily per calf or if the quality of hay fed at this stage had been better. Further study is required on the reasons for these results.

The practice of 'weaning' at 145 to 150 lb. liveweight appeared to produce better results than weaning by age. The lengths of time that 'milk' was fed varied from extremes of 28 days, for a calf weighing 110 lb. at birth, to 60 days for one weighing 65 lb. at birth. This method apparently permitted weaning at more nearly equal physiological ages or stages in the development of the digestive systems. The average age at weaning in this experiment was 45.5 days compared to a range of from 42 to 56 days in Experiment 1. The freedom from digestive disturbances, the uniformity of growth responses and the general thriftiness of calves justify the conclusion that, whereas weaning at 6 weeks of age involved problems with calves which were small at birth, an *average* weaning age of about 6 weeks was achieved successfully by weaning calves at weights of 145 to 150 lb.

ACKNOWLEDGEMENTS

The author acknowledges the assistance of herdsman George Stevens and D. McNaughton in feeding, weighing and caring for the calves.

The co-operation given by W. Wilson, Saskatchewan Federated Cooperatives, and by A. J. Bunn, Intercontinental Packers Limited, both of Saskatoon, in mixing milk replacer formulas is appreciated.

REFERENCES

1. Esh, G. C., T. S. Sutton, J. W. Hibbs, and W. E. Krauss. The effect of soya-phosphatides on the absorption and utilization of vitamin A in dairy animals. *J. Dairy Sci.* 31:4, 461-478. 1948.
2. Hopper, J. H., K. E. Gardner, and B. C. Johnson. Butyrate lard in the ad libitum feeding of "filled milk" for veal production. *J. Dairy Sci.* 37:4, 431-435. 1954.
3. Lassiter, C. A. Antibiotics as growth stimulants for dairy cattle. *J. Dairy Sci.* 38: 1102-1139. 1955.
4. Logan, V. S. Antibiotics in the feeding of dairy calves. *Agr. Inst. Rev.* 7:11-13. 1952.
5. Loosli, J. K., R. H. Wasserman, and L. S. Gall. Antibiotic studies with dairy calves. *J. Dairy Sci.* 34:500. 1951.
6. Matthews, C. A., and M. H. Fohrman. Beltsville growth standards for Holstein cattle. U.S.D.A. Tech. Bull. 1099. 1954.
7. Norton, C. L., and H. D. Eaton. Dry calf starters for dairy calves. Cornell University Agr. Expt. Sta. Bull. 835. 1946.
8. Recommended Nutrient Allowances for Dairy Cattle. National Research Council, Washington, D. C. 1945.
9. Reid, J. Thomas, Richard G. Warner, and John K. Loosli. Antibiotics in the nutrition of ruminants. *J. Agr. Food Chem.* 2:186-192. 1954.
10. Wallace, H. D., J. K. Loosli, and K. L. Turk. Substitutes for fluid milk in feeding dairy calves. *J. Dairy Sci.* 34:256-264. 1951.
11. Whiting, F., and R. D. Clark. Raising dairy calves with a limited amount of whole milk. *Can. J. Agr. Sci.* 35:454-460. 1955.

THE CALCIUM, PHOSPHORUS, AND ZINC BALANCE IN PIGS AS INFLUENCED BY THE WEIGHT OF PIG AND THE LEVEL OF CALCIUM, ZINC, AND VITAMIN D IN THE RATION¹

F. WHITING AND L. M. BEZEAU

Canada Department of Agriculture, Lethbridge, Alberta

[Received for publication June 12, 1958]

ABSTRACT

Digestion and metabolism experiments were carried out with pigs of various body weights (20, 35, 55, and 80 lb.) using 4 x 4 latin square designs of experiment to determine the effects of supplementing a ration with calcium, zinc, and vitamin D on the apparent absorption and retention of calcium, phosphorus, and zinc. The levels of calcium, zinc, and vitamin D in the rations were 0.40 and 0.86 per cent, 34 and 140 p.p.m., and 0 and 800 i.u. per lb. respectively. The pigs were hand-fed the rations in slop form.

Body weight gains and appetite were not affected by the addition of calcium, zinc, or vitamin D to the rations. Symptoms of parakeratosis appeared when the pigs were fed the basal or the basal-plus-calcium rations, but not when the rations containing supplemental zinc were fed.

The apparent absorption and retention of calcium were not influenced ($P=0.05$) by the addition of calcium or vitamin D to the ration or by the weight of pig, but were increased ($P<0.10$) by the addition of zinc to the ration. The apparent absorption of phosphorus was decreased ($P<0.01$) by the addition of calcium or vitamin D to the rations but was not influenced by the addition of zinc or by weight of pig. The retention of the phosphorus consumed was not affected by calcium, zinc, vitamin D, or weight of pig. The apparent absorption and retention of zinc were increased ($P<0.01$) by the addition of zinc, decreased ($P<0.01$) by the addition of vitamin D, and unaffected by the addition of calcium to the ration. The apparent absorption of zinc by the 20-lb. pigs was greater ($P<0.01$) than by the pigs of other weights and less ($P<0.05$) by the 35-lb. pigs than by the pigs of other weights. Most of the zinc consumed was excreted in the feces.

The results of this experiment indicate that a high vitamin D content of the ration and weight of pig may be of importance in the development of parakeratosis.

INTRODUCTION

The apparent interrelationship between calcium and zinc in influencing the severity of parakeratosis among pigs is well known (5, 21, 23, 25, 26, 32, 34). However, the method by which either calcium or zinc influences the absorption of the other, if such does occur, is not known with any certainty. Hoekstra *et al.* (18) reported that the addition of 2 per cent bonemeal to a zinc-supplemented ration did not significantly alter the zinc content of erythrocytes, blood plasma, liver, kidney, spleen, intestines, or pancreas. However, Lewis *et al.* (22, 23) found that increasing the calcium content of a ration resulted in an increase in the zinc content of the liver, kidneys, and hair, but no increase in the zinc content of the blood plasma, pancreas, skin, or intestines. Lewis *et al.* (21) also reported that injected zinc gave a similar response to dietary zinc in alleviating parakeratosis, indicating that the effect of zinc on calcium metabolism, or vice versa, was not confined to the gastro-intestinal tract. Hansard *et al.* (14) found a marked decrease in the net retention and in the apparent and true digestibility of calcium by sheep when they were fed additional zinc.

¹ Contribution from the Division of Animal Husbandry, Experimental Farms Service, Canada Department of Agriculture.

The digestibility and retention of zinc by the pig have not been studied extensively. Studies with rats have indicated that the absorption of zinc from the gastro-intestinal tract is poor (8, 9, 15).

Vitamin D has been shown to influence the absorption of calcium from the digestive tract of poultry and mammals and its utilization within the body (6, 7, 19, 28). It is possible, also, that vitamin D may influence the availability or metabolism of zinc if it is shown that calcium influences the availability or metabolism of zinc.

Digestion and metabolism experiments were carried out at the Experimental Farm, Lethbridge, Alberta, with pigs of various body weights to determine the effects of supplementing a ration with calcium, zinc, and vitamin D on the apparent digestibility and retention of calcium, phosphorus, and zinc. The purpose of this experiment was to learn whether parakeratosis in pigs could be explained on the basis of the influence of calcium or zinc on the absorption and retention of the other.

PROCEDURE

Eight male castrated pigs of Berkshire \times Lacombe breeding were used in digestion and metabolism studies, using two 4 \times 4 latin square designs of experiment. The pigs were weaned from the sows at 4 weeks of age and placed on experiment. Within the first latin square four rations (no supplement, calcium, zinc, and calcium and zinc added) and four weights of pigs (20, 35, 55, and 80 lb.) were studied. Within the second latin square the same rations and weights of pigs were used, except that the rations were supplemented with 800 i.u. vitamin D per lb. of ration. The four pigs in each latin square were litter mates; those in the first latin square were white in colour and those in the second were black. Because the pigs used in the two latin squares were from different litters, the effect of vitamin D was confounded with litters. However, since all pigs were closely related, it is not expected that there would be any differences between litters in their utilization of minerals.

Each experimental period was 28 days (18 days preliminary and 10 days collection). During the first 8 days of the preliminary period, maximum feed consumption was determined and thereafter this amount was fed daily during the remaining 20 days in two equal feedings at approximately 8 a.m. and 4 p.m. To minimize scattering of the feed by the pigs, each day's feed was mixed with three times its weight of 'demineralized' water. Each pig was used during four successive periods and weighed approximately 20, 35, 55, and 80 lb. during these periods. Metabolism cages similar to those described by Bell (4) were used. To avoid contamination with zinc, polyethylene urine collection bottles and aluminum feeders were used, and all metal surfaces on the cages were coated with a plastic-type paint.

A semi-purified basal ration was used in an attempt to have a low zinc content and was made up as follows (in parts per 100): Corn starch, 40; sucrose, 5; ground oats, 20; alfalfa meal, 10; soybean oilmeal, 20; fishmeal, 4; mineral mix, 1; vitamin mix, 0.1. The mineral mix was composed of: NaCl, 47 lb.; calcium phosphate*, 47 lb.; KCl, 5 lb.; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 2 lb.;

* The calcium phosphate (20% P and 27% Ca) was supplied by N. D. Hogg Ltd., Toronto, Ont.

MnSO₄, 90 gm.; CuSO₄, 90 gm.; CuCl₂ 20 gm.; and KI, 20 gm. The vitamin mix was composed of vitamin B mixture* (36), 1.16 gm.; Aurofac 10*, 23 gm.; Profactor B*, 23 gm.; and vitamin A concentrate, 8 gm. (10,000 i.u. per gm.).

The basal ration contained 15.6 per cent protein (N x 6.25), 0.40 per cent calcium, 0.46 per cent phosphorus, and 34 p.p.m. zinc (air-dry basis). Eight rations were formulated to contain two levels of calcium, two levels of zinc, and two levels of vitamin D by adding ground limestone, zinc sulphate, and irradiated dry yeast*. The calcium, zinc, and vitamin D content of the rations were as follows:

	Calcium %	Zinc (p.p.m.)	Vitamin D i.u./lb.
Ration A (basal)	0.40	34	0
B	0.86	34	0
C	0.40	140	0
D	0.86	140	0
E	0.40	34	800
F	0.86	34	800
G	0.40	140	800
H	0.86	140	800

The zinc contained in the water consumed was added to that consumed in the ration. This water contained 1.7 p.p.m. of zinc.

Analysis of variance was used to determine whether differences caused by the various additions to the rations on weights of pig were significant. Percentage data were transformed to angles (31) before analysing them.

The wet ashing and colorimetric methods of the A.O.A.C. (2) were used in the analyses of feed, feces, and urine for calcium, phosphorus, and zinc. The vitamin D content of the rations was not determined.

RESULTS AND DISCUSSION

The average daily body weight gains and the average apparent percentage absorption and retention of calcium and phosphorus by pigs as influenced by the calcium, zinc, and vitamin D content of the ration are shown in Table 1. Weight of pig had no influence ($P=0.05$) on the absorption and retention of calcium and phosphorus and is not shown in tabular form. Similar data for zinc but showing the effect of weight of pig on apparent absorption and retention of zinc are shown in Table 2.

Incidence of Parakeratosis and Body Weight Gains

Parakeratotic skin lesions occurred among the pigs fed the basal and the basal-plus-calcium rations. The lesions were more noticeable among

* The B-vitamin mixture by Hoffman-LaRoche Inc., Nutley, N.J.; Aurofac 10 (contains at least 10 gm. Chlortetracycline per lb.) and the Profactor B (contains at least 10 mg. vitamin B₁₂ per lb.), by Lederle Laboratories, Pearl River, N.Y.; irradiated dry yeast by Standard Brands, Montreal, Que.

TABLE 1.—THE AVERAGE DAILY BODY WEIGHT GAINS AND THE AVERAGE APPARENT PERCENTAGE ABSORPTION AND RETENTION OF CALCIUM AND PHOSPHORUS BY THE PIG AS INFLUENCED BY THE CALCIUM, ZINC, AND VITAMIN D CONTENT OF THE RATION

	Additions to basal ration							
	No vitamin D				Vitamin D			
	O	Ca	Zn	Ca & Zn	O	Ca	Zn	Ca & Zn
Average daily body gain (lb.)	0.64	0.73	0.69	0.60	0.79	0.71	0.78	0.73
<i>Calcium</i> —								
absorbed (apparent)	52	53	46	53	41	43	58	52
retained of consumed	48	45	42	45	35	35	53	44
retained of absorbed	92	86	91	85	86	80	91	84
excreted in urine	4	7	4	8	5	8	5	9
<i>Phosphorus</i> —								
absorbed (apparent)	61	54	59	52	54	45	56	48
retained of consumed	52	52	51	51	47	45	45	45
retained of absorbed	86	98	87	99	85	99	83	98
excreted in urine	10	1	8	1	8	0	9	1

the pigs during the second period (i.e. 35-lb. pigs) and among those not receiving vitamin D. However, since the colour of the skin and hair of the pigs receiving vitamin were black and those not receiving vitamin D were white (see under Procedure), the parakeratotic skin lesions were not as evident on the black pigs; hence, it is possible that the skin colour and not vitamin D was the more important factor in judging the severity of skin lesions. The skin conditions cleared up when the pigs were fed rations containing supplemental zinc.

Body weight gains and appetites of the pigs were not affected by the various experimental rations (Table 1). The pigs showing marked skin lesions consumed their daily ration and made body weight gains equal to those that showed no lesions. It has been reported that decreased gains and a poor appetite are the first symptoms of parakeratosis (3, 20, 21, 32). Hoefer (17) reported that pigs fed high-calcium rations (1.25 per cent calcium) without supplemental zinc gained very poorly as compared with those fed the same quantity of feed but with additional zinc. However, it has been reported also that high-calcium rations have been fed with and without supplemental zinc with no differences noted in body weight gains (1).

It should be emphasized that, in the experiment reported here, the pigs were hand-fed their ration in slop form. A number of reports have indicated that symptoms of parakeratosis (both skin and body weight changes) were more severe when the pigs were self-fed a dry ration than when hand-fed a slop ration, (5, 20, 24, 33). However, the fact that parakeratotic skin lesions did develop in the pigs in this experiment indicates that parakeratosis can be produced among pigs hand-fed a slop ration. It also indicates that any interrelationships found between calcium, zinc, and vitamin D in this experiment should be valid also for pigs self-fed a dry ration.

TABLE 2.—THE PERCENTAGE ABSORPTION (APPARENT) AND RETENTION OF ZINC BY PIGS OF VARIOUS BODY WEIGHTS AS INFLUENCED BY THE CALCIUM, ZINC, AND VITAMIN D CONTENT OF THE RATION

	Weight of pig (lb.)	Additions to the basal ration							
		No vitamin D				Vitamin D			
		O	Ca	Zn	Ca & Zn	O	Ca	Zn	Ca & Zn
Absorbed	20	50	39	68	64	26	30	61	60
	35	23	19	44	39	-7	-6	42	48
	55	18	24	56	36	15	24	40	64
	80	28	24	44	51	18	15	46	35
	Av.	30	26	53	49	13	16	47	52
Retained of consumed	20	41	24	62	58	10	8	52	52
	35	6	0	37	31	-25	-23	42	43
	55	8	12	51	33	6	14	36	60
	80	19	14	40	47	6	-2	41	29
	Av.	18	12	48	42	-1	-1	40	46
Retained of absorbed	20	82	60	90	90	39	28	85	87
	35	28	-2	84	79	-1	-1	88	91
	55	48	49	92	89	37	58	91	93
	80	68	57	91	92	32	-12	89	83
	Av.	56	41	89	8	6	2	88	88
Excreted in urine	20	10	19	7	7	16	22	9	8
	35	19	23	7	8	17	17	5	4
	55	11	14	4	4	9	10	4	5
	80	10	16	4	4	12	16	5	6
	Av.	12	18	6	6	14	16	6	6

¹ Since the percentage absorption was negative, the values for percentage retention of that absorbed are meaningless and not reported. Averages were evaluated from the total retained of that absorbed by the 4 pigs.

The Apparent Absorption and Retention of Calcium

The apparent absorption and the retention of the calcium consumed were not influenced ($P=0.05$) by the addition of calcium or vitamin D to the ration or by weight of pig. The addition of zinc to the ration increased ($P<0.10$) the average apparent absorption of calcium from 47 per cent to 52 per cent and the retention from 41 per cent to 46 per cent. There was a significant interaction ($P<0.05$) between zinc and vitamin D on both the apparent absorption and retention of calcium. In the absence of zinc, the addition of vitamin D decreased the absorption and retention of calcium and in the presence of zinc, vitamin D increased the absorption and retention of calcium. The significance that this interaction may have on the development of parakeratosis was not directly evident, but it may assist in explaining why supplemental vitamin D has increased calcium absorption in some experiments and decreased it or had no effect in other experiments.

The percentage retention of the apparently absorbed calcium was less at the higher level than at the lower level of calcium intake (84 vs. 90 per cent). The addition of zinc or vitamin D, or the weight of pig, did not influence the retention of the apparently absorbed calcium. Hansard and Plumlee (13) have shown with rats that the body stores of calcium mater-

ially affect the absorption of calcium from the intestinal tract and the excretion into the tract (endogenous fecal calcium). Hence, it was not possible to calculate the true absorption of calcium in the present experiment, and emphasis was placed on the net retention of calcium.

On the basis of studies with rats (12, 16) and cattle (11), one would have expected the 20-lb. pigs to have absorbed and retained a greater percentage of the dietary calcium than found in this experiment and also a greater percentage than the 35-, 55-, and 80-lb. pigs. Since a high calcium intake is thought to increase the incidence of parakeratosis, and zinc to decrease it, it was expected also that the addition of zinc would decrease the absorption of calcium as has been reported for sheep (14). However, such was not the case in this experiment. Vitamin D has been reported to increase the absorption of calcium in the normal rat (10), rachitic rat (35), 6-months-old Hereford calf (6), and chicken (19, 28). On the other hand, Henry and Kon (16) reported that vitamin D had no effect on the calcium metabolism of rats fed rations containing 0.25 and 0.8 per cent calcium.

The Apparent Absorption and Retention of Phosphorus

The apparent absorption of phosphorus was decreased ($P < 0.01$) by the addition of calcium or vitamin D to the ration, but was not influenced by the addition of zinc or by the weight of the pig. There was a tendency for the digestibility of the phosphorus to decrease as the pigs became heavier. The retention of the phosphorus consumed was not influenced by the addition of calcium or zinc to the ration but was reduced ($P < 0.01$) by the addition of vitamin D to the ration. The retention of the absorbed phosphorus was increased ($P < 0.01$) by the addition of calcium to the ration but was not affected by the addition of zinc and vitamin D or the weight of the pig. There was a significant interaction ($P < 0.05$) between zinc and vitamin D in influencing the retention of digested phosphorus. In the absence of vitamin D, zinc increased the retention of phosphorus, whereas, in the presence of vitamin D, zinc decreased the retention of phosphorus.

Less phosphorus was excreted in the urine at the higher level of calcium intake than at the lower level (average 0.03 vs. 0.32 gm. daily). Hansard and Plumlee (13) noted that rats receiving 0.013 per cent calcium and 0.4 per cent phosphorus in their ration retained less phosphorus than those receiving a ration containing 0.5 per cent calcium and 0.4 per cent phosphorus. Apparently, on the low-calcium diet a large part of the phosphorus was absorbed but was immediately excreted in the urine. Although the calcium contents of the diets used by Hansard and Plumlee were more extreme than those used in the present experiment, the trend was the same.

The Apparent Absorption and Retention of Zinc

The percentage apparent absorption and retention of zinc were increased ($P < 0.01$) by the addition of zinc, decreased ($P < 0.01$) by the addition of vitamin D, and unaffected by the addition of calcium to the ration. These findings indicate that, if parakeratosis is caused by a zinc deficiency, the addition of calcium in this experiment played no part in influencing either the apparent absorption or retention of zinc. Beardsley and Forbes

(3) have reported that the level of calcium in the ration did not influence the severity of parakeratosis among pigs 2 to 8 weeks old when fed semi-purified rations. However, as mentioned previously, a number of workers have reported that level of calcium in the ration influences the severity of parakeratosis (5, 21, 23, 25, 26, 32, 34). Bellis and Phelps (5) reported that a reduction of calcium or an increase of phosphorus in the ration decreased the dermatitis in pigs but did not improve growth or feed consumption. Lewis *et al.* (24) used *in vitro* experiments and found that the calcium: phosphorus ratio and pH affected the removal of zinc from solution. Newland *et al.* (29) found that, when the calcium content of a ration (zinc content 30 p.p.m.) was increased from 0.64 to 1.19 per cent, the endogenous fecal zinc content was increased.

Weight of pig also affected ($P < 0.01$) the apparent absorption of zinc. The high absorption (apparent) of zinc by the 20-lb. pigs and the low absorption by the 35-lb. pigs as compared to the 55- and 80-lb. pigs may explain why the incidence of parakeratosis is apparently low among very young pigs (birth to 25 lb.), highest among pigs 30 to 50 lb., and then lessens as the pigs become older, and why pigs later often recover without therapy.

There were significant interactions in the absorption and retention of zinc between levels of zinc and vitamin D intakes ($P < 0.01$), weights of pigs and zinc intake ($P < 0.05$), and weights of pigs and vitamin D intake ($P < 0.05$). The latter interaction was significant for absorption but not for retention of zinc. When vitamin D was added to the rations containing no supplemental zinc, it decreased the absorption and retention of zinc but had no effect when the rations contained supplemental zinc. The interaction between weights of pigs and zinc intake was caused by the very low absorption and the negative retention of zinc by the 35-lb. pigs when fed the rations containing no supplemental zinc but almost as high an absorption and retention of zinc by the 35-lb. pigs as the other weights of pigs when supplemental zinc was fed. The interaction between vitamin D intake and weights of pigs was caused by the lower absorption and retention of zinc by the 35-lb. pigs when vitamin D was added to the ration than when vitamin D was not added.

Approximately 80 per cent of the zinc consumed in the feed and water by the pigs fed the rations without supplemental zinc was excreted in the feces. When the zinc-supplemented rations were fed, only 50 per cent was excreted in the feces. This indicates either that the zinc added to the ration as zinc sulphate was more highly digestible than zinc occurring in the various feedstuffs used or that a considerable amount of endogenous zinc was excreted in the feces. Skeline *et al.* (30) reported that dogs and mice excrete a large fraction of body zinc through the gastro-intestinal tract and, as mentioned above, Newland *et al.* (29) found that, when the calcium content of the ration was increased from 0.64 to 1.19 per cent, the endogenous fecal zinc also was increased (zinc content of the ration was 30 p.p.m.). The low absorption of zinc reported here agrees with other published data (8, 15, 27).

Approximately 15 per cent of the zinc consumed by pigs fed the rations without supplemental zinc was excreted in the urine, and about 6 per cent of that consumed when the pigs were fed the zinc-supplemented rations.

However, the daily weight of zinc excreted in the urine by the pigs fed supplemental zinc was about 50 per cent more than that excreted by the pigs not receiving supplemental zinc. Heller and Burke (15) reported that zinc content of the urine was slightly increased when the animals (rats) were given additional zinc, but McCance and Widdowson (27) reported that the urinary excretion of zinc was not affected by dietary intake.

When the rations that were not supplemented with zinc were fed, the pigs retained an average of 2.5 mg. of zinc daily (0.2 mg. for those receiving vitamin D and 4.9 mg. for those not receiving vitamin D), and when zinc-supplemented rations were fed the pigs retained an average of 54 mg. (52 and 56 mg. for pigs receiving and not receiving supplemental vitamin D, respectively).

The results from this experiment do not indicate why the incidence of parakeratosis, if caused by a high calcium intake, is reduced by feeding additional zinc. In this experiment supplemental zinc increased ($P < 0.10$) the absorption and retention of calcium, the opposite to what might be expected, and supplemental calcium had no effect on the absorption or retention of zinc.

A high vitamin D intake may be involved in parakeratosis in that it reduced the apparent absorption and the retention of zinc when no supplemental zinc was fed. The effect of size of pig on the absorption and retention of zinc may be of some importance in the development of parakeratosis.

REFERENCES

1. Anonymous 80th Ann. Rept., Ontario Agr. Coll., p. 24. 1955.
2. Association of Official Agricultural Chemists. Official methods of analysis. 8th ed. Washington, D.C. 1955.
3. Beardsley, D. W., and R. M. Forbes. Growth and chemical studies of zinc deficiency in the baby pig. *J. Animal Sci.* 16:1038. 1957. (Abstr.).
4. Bell, J. M. An adjustable cylindrical cage for use in metabolism studies with young pigs. *J. Nutrition* 35:365-369. 1948.
5. Bellis, D. D., and J. McL. Philp. Effect of zinc, calcium, and phosphorus on the skin and growth of pigs. *J. Sci. Food Agr.* 8:5119-5127. 1957. (Suppl. issue).
6. Conrad, H. R., and Sam L. Hansard. Effects of massive doses of vitamin D on physiological behavior of calcium in cattle. *J. Applied Physiol.* 10:98-102. 1957.
7. Cramer, John W., and Harry Steenbock. Calcium metabolism and growth in rats on a low-phosphorus diet as affected by vitamin D and increases in calcium intake. *Arch. Biochem. and Biophys.* 63:9-13. 1956.
8. Feaster, J. P., Sam L. Hansard, J. T. McCall, and G. K. Davis. Absorption, deposition, and placental transfer of zinc 65 in the rat. *Amer. J. Physiol.* 181:287-290. 1955.
9. Gilbert, I. C. F., and D.M. Taylor. The behavior of zinc and radio-zinc in the rat. *Biochem. biophys. Acta* 21:545-551. 1956. (Abstracted in *Nutrition Abstr. & Revs.* 27:477. 1957.)
10. Haavaldsen, Ragnar, and Ragnar Nicolaysen. Calcium metabolism in rats. 1. Long term study in rats given an optimal diet with and without vitamin D. *Acta Physiol. Scand.* 36:102-107. 1956. (Abstracted in *Chem. Abstr.* 50:14899. 1956.)
11. Hansard, Sam. L., C. L. Comar, and M. P. Plumlee. The effects of age upon calcium utilization and maintenance requirements in the bovine. *J. Animal Sci.* 13:25-36. 1954.
12. Hansard, Sam L., and H. M. Crowder. The physiological behavior of calcium in the rat. *J. Nutrition* 62:325-339. 1957.
13. Hansard, Sam L., and M. P. Plumlee. Effect of dietary calcium and phosphorus levels upon the physiological behavior of calcium and phosphorus in the rat. *J. Nutrition* 54:17-31. 1954.

14. Hansard, Sam L., Alan Thompson, and M. C. Bell. The metabolism of calcium and phosphorus as affected by zinc and aluminum in rations for sheep. *J. Animal Sci.* 16:1094. 1957. (Abstr.).
15. Heller, V. G., and A. D. Burke. Toxicity of zinc. *J. Biol. Chem.* 74:85-93. 1927.
16. Henry, Kathleen M., and S. K. Kon. The relationship between calcium retention and body stores of calcium in the rat: Effect of age and of vitamin D. *Brit. J. Nutrition* 7:147-159. 1953.
17. Hoefler, J. A. Parakeratosis in swine. *Proc. Cornell Nutrition Conf. for Feed Manufacturers*, pp. 37-43. 1956.
18. Hoekstra, W. G., P. K. Lewis, Jr., P. H. Phillips, and R. H. Grummer. The relationship of parakeratosis, supplemental calcium, and zinc to the zinc content of certain body components of swine. *J. Animal Sci.* 15:752-764. 1956.
19. Keane, K. W., R. A. Collins, and M. B. Gillis. Isotopic tracer studies on the effect of vitamin D on calcium metabolism in the chick. *Poultry Sci.* 35:1216-1222. 1956.
20. Kuit, A. R. An outbreak of suspected zinc deficiency (parakeratosis) in pigs. *Tijdschr Diergeneesk.* 81:698-704. 1956. (Abstracted in *Vet. Bull.* 27:193. 1957.)
21. Lewis, P. K., Jr., W. G. Hoekstra, R. H. Grummer, and P. H. Phillips. The effect of certain nutritional factors including calcium, phosphorus, and zinc on parakeratosis in swine. *J. Animal Sci.* 15:741-751. 1956.
22. Lewis, P. K., Jr., W. G. Hoekstra, and R. H. Grummer. The action of high calcium and self-feeding in aggravating parakeratosis. *J. Animal Sci.* 15:1265-1266. 1956. (Abstr.).
23. Lewis, P. K., Jr., W. G. Hoekstra, and R. H. Grummer. Restricting calcium feeding versus zinc supplementation for the control of parakeratosis in swine. *J. Animal Sci.* 16:578-588. 1957.
24. Lewis, P. K., Jr., R. H. Grummer, and W. G. Hoekstra. The effect of method of feeding upon the susceptibility of pigs to parakeratosis. *J. Animal Sci.* 16:927-936. 1957.
25. Luecke, R. W., J. A. Hoefler, W. S. Brammell, and F. Thorpe, Jr. Mineral interrelationships in parakeratosis of swine. *J. Animal Sci.* 15:347-352. 1956.
26. Luecke, R. W., J. A. Hoefler, W. S. Brammell, and D. A. Schmidt. Calcium and zinc in parakeratosis of swine. *J. Animal Sci.* 16:3-11. 1957.
27. McCance, R. A., and E. M. Widdowson. The absorption and excretion of zinc. *Biochem. J.* 36:692-696. 1942.
28. Migicovsky, B. B. Influence of vitamin D on calcium resorption and accretion. *Can. J. Biochem. and Physiol.* 35:1267-1275. 1957.
29. Newland, H. W., D. E. Ullrey, J. A. Hoefler, and R. W. Luecke. The relationship of dietary calcium to zinc metabolism in pigs. *J. Animal Sci.* 15:1250-1251. 1956. (Abstr.).
30. Skeline, G. E., I. L. Charkoff, H. B. Jones, and M. Lawrence Montgomery. Studies on the metabolism of zinc with the aid of its radioactive isotope. I. The excretion of administered zinc in urine from feces. *J. Biol. Chem.* 147:409-414. 1943.
31. Snedecor, George W. *Statistical method*. 5th ed. Iowa State College Press, Ames, Iowa. 1956.
32. Stevenson, J. W., and I. P. Earle. Studies on parakeratosis in swine. *J. Animal Sci.* 15:1036-1045. 1956.
33. Thomas, G., and A. Eden. A peculiar dermatitis in pigs. *Nature* 174:553. 1954.
34. Tucker, Howard F., and W. D. Salmon. Parakeratosis or zinc-deficiency disease in the pig. *Proc. Soc. Exptl. Biol. Med.* 88:613-616. 1955.
35. Underwood, Elizabeth, Solomon Fisch, and Harold C. Hodge. Metabolism of calcium in normal, rachitic, and vitamin-D treated rats as evidenced by radio-calcium Ca^{45} studies. *Amer. J. Physiol.* 166:387-393. 1951.
36. Whiting, F., and L. M. Bezeau. The nutritional value of frost-damaged and early-harvested cereal grains for swine. *Can. J. Agr. Sci.* 34:624-634. 1954.

THE SPARING EFFECT OF WHEAT ON THE NEED FOR PROTEIN CONCENTRATES IN A TURKEY STARTING RATION

B. E. MARCH AND J. BIELY

*Poultry Nutrition Laboratory, The University of British Columbia,
Vancouver, British Columbia*

[Received for publication March 30, 1958]

ABSTRACT

Wheat and corn respectively were used as the principal cereal in turkey poult starting rations. The results of three experiments indicate that advantage may be taken of the relatively high protein content of wheat. By using wheat and taking into consideration the additional protein thus provided, a saving may be effected in the amount of supplementary protein required. Turkey poults grew at similar rates when fed starting rations of similar content whether the main cereal constituent of the rations was wheat or corn.

INTRODUCTION

In formulating poultry rations, little or no allowance is currently made for the relatively high protein content of wheat as opposed to corn or for differences in the protein content of different samples of wheat. The average protein content of wheat produced in Western Canada in the years 1937 to 1956 was 13.5 per cent (5). There was a considerable range in protein content among regions and among years. In view of the probability that some of the individual amino acids (in addition to methionine which is already used extensively) will become available as supplements for animal feeds, the question of utilizing to best advantage the protein content of wheat is important.

In a study in which wheat samples of different protein content were fed to chicks in diets containing no supplementary protein, March, Biely and Tonzetich (3) found that growth rate and feed efficiency were slightly better with wheat of high protein content. High protein wheat (16.5 per cent), however, gave slower growth and poorer feed efficiency than wheat of lower protein content (11-13 per cent), when supplemented with fishmeal in chick starting rations. Biely *et al.* (2) showed that wheat could be substituted for corn on a pound-for-pound basis without reducing the growth response to the ration. In the latter study it was shown that, without specific amino acid supplementation, full advantage could not be taken of high protein wheat in broiler rations. It was thought, however, that it might be possible to utilize wheat protein more effectively in turkey rations. In poult rations the relative proportion of supplementary protein to cereal protein is considerably higher than in chick rations. Consequently, in poult rations, there might be a sufficient excess of lysine contributed by the customary protein concentrates to make up the lysine deficiency in the wheat protein.

MATERIALS AND METHODS

The composition of the diets is given in Table 1. The only ingredients which were varied in the different diets were wheat, corn and soybean oil

TABLE 1.—COMPOSITION OF DIETS

	Corn diet lb./100 lb.	Wheat diet lb./100 lb.	N.R.C. ¹ requirement
Ground yellow corn	40.0	—	
Ground wheat	—	46.74	
Soybean oil meal	25.3	18.56	
Ground oats	5.0	5.0	
Dehydrated cereal grass	3.0	3.0	
Herring meal	10.0	10.0	
Meatmeal	5.0	5.0	
Dried skim milk	2.5	2.5	
Dried corn fermentation solubles	3.0	3.0	
Dried brewers' yeast	2.0	2.0	
Limestone	2.0	2.0	
Bonemeal	1.0	1.0	
Iodized salt	0.5	0.5	
Feeding oil (2250 units A—300 units D)	0.5	0.5	
Choline chloride 25%	0.2	0.2	
Manganese sulphate	6.0 grams	6.0 grams	
α-alpha-tocopheryl acetate	0.25 “	0.25 “	
Chlortetracycline HCl	2.5 “	2.5 “	
Protein (analysed) %	27.7	28.6	28.0
Arginine (calculated) %	1.68	1.57	1.6
Lysine “ %	1.83	1.70	1.5
Methionine “ %	0.54	0.54	0.52
Cystine “ %	0.43	0.44	0.35
Tryptophan “ %	0.34	0.34	0.26

¹ National Research Council, Washington, D.C.

meal. Rations 2 and 4 were formulated from 1 and 3 respectively by the addition of 8 lb. of animal fat* per 100 lb.

The poults were reared in electrically heated battery brooders for 3 weeks. At 3 weeks they were transferred to unheated batteries. Feed and water were given *ad libitum*.

Experiment 1—Day-old Broad Breasted Bronze male poults were fed a commercial poult starter for 4 days. They were then wing-banded, weighed and fed the experimental rations. The birds were individually weighed when they were 53 days old. Record was kept of feed consumption.

Experiment 2—Day-old Broad Breasted Bronze male poults were fed the experimental diets. They were individually weighed at 42 days. Record was kept of feed consumption.

Experiment 3—Two different samples of wheat containing 14.3 and 12.7 per cent protein respectively were compared with corn as the principal cereal component in diets of similar composition to those in Experiments 1 and 2. Slight adjustments were necessary in the level of soybean oil meal in the diets.

Each diet was fed to duplicate lots of male and female Beltsville Small White turkey poults to 5 weeks of age. Approximately 92 poults were fed each experimental diet. They were individually weighed and record was kept of feed consumption.

* Sta-Y-fat. (Gordon Young (B.C.) Ltd., Vancouver, B.C.) A product stabilized with not more than 0.02 per cent butylated hydroxyanisole, 0.01 per cent propyl gallate and 0.004 per cent citric acid.

TABLE 2.—AVERAGE WEIGHTS OF POULTS IN EXPERIMENTS 1 AND 2

Characteristic of diet	Experiment 1		Experiment 2	
	Av. wt. (gm.) at 53 days	Feed Gain	Av. wt. (gm.) at 42 days	Feed Gain
Corn (8.6% protein)	1169	2.91	880	2.46
Corn, fat supplement	1122	2.78	870	2.34
Wheat (13.7% protein)	1195	3.11	986	2.38
Wheat, fat supplement	1201	2.82	863	2.42

TABLE 3.—AVERAGE WEIGHTS OF POULTS IN EXPERIMENT 3 AT 5 WEEKS OF AGE

Characteristic of diet	Males	Females	Feed/Gain males and females
Corn (8.6% protein)	792	723	1.94
Wheat (14.3% protein)	806	738	1.92
Wheat (12.7% protein)	838	739	1.97

RESULTS AND DISCUSSION

The amino acid composition of the diets as calculated from the values compiled by Almquist (1) are shown in Table 1. In the diets with added tallow these values will be 8 per cent lower on the basis of the total ration but would be unchanged if calculated on a protein basis. By comparison with the requirements given by the National Research Council (4), both the diets should have been adequate in the essential amino acids considered. In the wheat diets the substitution of wheat protein for some of the soybean oil meal protein lowered the levels of lysine and arginine. As a result of the high proportion of supplementary protein to cereal protein the levels were not, however, below the National Research Council recommendations although the level of arginine approached the listed requirement.

In Experiments 1 and 2 the growth rates (Table 2) of the poults fed the wheat and the corn diets were similar. There were no significant differences in the average weights of the poults fed the diets with or without supplementary fat. Furthermore, the poults fed the diets to which 8 per cent of fat was added grew at the same rate whether the diet contained wheat or corn.

The results of Experiment 3 are shown in Table 3. They indicate that the extra protein contained in the high protein wheat, as compared with the wheat of lower protein content, was used effectively to replace an

equivalent amount of protein from soybean oil meal. Similarly, the additional protein contained in each of the samples of wheat tested over corn was likewise able to replace an equivalent amount of protein from soybean oil meal.

The data on feed utilization show that there was little difference in the efficiency with which the wheat and the corn diets were utilized. This is of interest inasmuch as previous studies in connection with the use of wheat versus corn in poultry rations have generally indicated that, when wheat is substituted for corn, there is a decrease in feed efficiency even when growth is unaffected. Usually, however, the wheat has been used to replace corn on a pound-for-pound basis without adjustment for the difference in the protein content of the two grains.

From the results of the experiments it is concluded that the high protein content of wheat as compared to corn may be used to advantage in turkey poult rations to take the place of some supplementary protein. This finding is in contrast to that in previous experiments with chick rations in which the high protein content of wheat had to be discounted in order to supply sufficient protein from supplementary sources to make up the amino acid deficiencies in wheat.

REFERENCES

1. Almquist, H. J. *Proteins and amino acids in animal nutrition*. 3rd ed. Grange Co., Modesto, Calif.
2. Biely, J., B. E. March, G. L. Inkin, and G. T. Eedy. Wheat versus corn in the Connecticut broiler ration. *Poultry Sci.* 30:595-598. 1951.
3. March, B., Jacob Biely, and J. Tonzetich. Supplementation of wheat with amino acids in the diet of the chick. *J. Nutrition* 42:565-575. 1950.
4. *Nutrient requirements for poultry*. Publ. 301, National Research Council, Washington, D.C. 1954.
5. *Protein Survey of Wheat*. Crop Bull. 66, Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, Man. 1957.

TRICHOLOMA NUDUM AS A SOURCE OF AMINO ACIDS, VITAMINS AND UNIDENTIFIED FACTORS

J. M. BELL,¹ J. D. ERFLE,² J. F. T. SPENCER³ AND F. REUSSER⁴

Saskatoon, Saskatchewan

[Received for publication July 28, 1958]

ABSTRACT

Feeding trials with mice were used to evaluate the nutritional quality of dried mycelia from *Tricholoma nudum* grown in submerged culture. The material proved to be non-toxic at dietary levels as high as 50 per cent. Chemical and bio-assays indicated methionine and phenylalanine to be the first and possibly the only limiting amino acids when *Tricholoma* supplied all the dietary protein at a level of 18 per cent. The product was relatively rich in arginine and tryptophane.

Tricholoma residue, at a level of 5 per cent in the diet, provided all the necessary B-vitamins for normal growth in the mouse. Chemical or microbiological assays were obtained for riboflavin, niacin, pantothenic acid, choline and thiamin and, in all cases except niacin, the levels exceeded those reported for average *Torula* yeast.

Tricholoma was found to possess a factor having strong antioxidant properties. The inclusion of 10 per cent *Tricholoma* in the diet was more effective in preventing a rise in peroxide values than was 0.05 per cent DPPD.

INTRODUCTION

Previous work (6) indicated that the dried mycelium from submerged culture of *Tricholoma nudum* had potentially good feeding value. Protein and amino acid analyses and toxicity tests were reported.

This communication reports further information on the biological value of the protein and the value of the product as a source of B-vitamins and of a factor affecting the development of rancidity in dietary fat.

MATERIALS AND METHODS

Tricholoma nudum was grown in submerged culture in a molasses medium (6) and subsequently freeze-dried for use in feeding trials with mice. The material was light grey in colour, very light textured and of pleasant, mild aroma.

Chemical Analyses

The mycelium was analysed for crude protein (Nx6.25), crude fat, total ash and moisture according to standard procedures for feedingstuffs analysis. Carbohydrate content (expressed as glucose) was determined colorimetrically by the anthrone method (5). Essential amino acids were determined quantitatively by one-dimensional ascending paper chromatography (4,7). Peroxides were determined by the method of Sulley (9).

Vitamin assays were obtained for riboflavin, niacin, pantothenic acid (total and free), thiamin and choline.

¹ Professor, Head, Department of Animal Husbandry, University of Saskatchewan, Saskatoon, Sask.

² Graduate Assistant, Department of Animal Husbandry, University of Saskatchewan, Saskatoon, Sask.

³ Assistant Research Officer, Engineering and Process Development Section, National Research Council, Saskatoon, Sask.

⁴ Formerly Post-doctorate Fellow, National Research Council, Saskatoon, Sask.

Animal Feeding Tests

The mouse experiments were conducted in two phases. The first was an attempt to evaluate the amino acid and vitamin B-complex content of the product. The second experiment was an investigation of an apparent relationship between the presence of the mycelium and the development of rancidity in the fat component of the diets since diets devoid of the mycelium proved to contain rancid fat at the end of the 14-day feeding tests.

Experiment No. 1

In this test weanling female mice of Carworth Farms No. 1 strain were allotted randomly to the various diets and were housed in individual wire-bottom, metal cages in a mobile battery. The laboratory was thermostatically controlled at about 25° C. At the time of allotment the mice weighed between 8.0 and 9.0 gm. and were under 23 days of age. Feed and water were provided *ad libitum* and accurate records of feed were kept during a 14-day growth test.

The experiment included nine diets. *Diet 1*, the stock colony diet, had proven satisfactory over several years of use in this laboratory and was, therefore, used as a positive control ration. It was composed of wheat 15.0; oat groats 20.0; wheat bran 15.0; barley 12.0; alfalfa leaf meal 5; linseed oilmeal 7.5; meat meal 15; molasses 5; brewers' dried yeast 1; lard (emulsified and stabilized) 2.5; ground limestone 1, and iodized salt 0.5 per cent, plus 10 p.p.m. of aureomycin, 250 p.p.m. manganese sulphate, 5000 I.U. vitamin A per pound of feed and 70 I.U. vitamin D₃ per pound of feed.

All other diets were semi-purified and designed to contain approximately 16 per cent protein together with adequate levels of minerals, digestible energy and vitamins in accordance with known nutrient requirements for the mouse or rat. *Diet 2* was corn starch 45; sucrose 10; cellulose¹ 10; minerals² 5; corn oil 5; vitamin-free casein 20 and vitamin mixture 5 per cent. The vitamin mixture contained 0.3 mg. thiamin; 0.4 mg. riboflavin; 0.9 mg. pantothenic acid; 3 mg. niacin; 10 μ gm. biotin; 0.1 mg. pyridoxine; 0.5 μ gm. B₁₂; 25 μ gm. tolacin; 0.1 mg. inositol; 85 mg. choline; 50 I.U. vitamin A; 0.5 μ gm. vitamin D₂; 7 I.U. α -tocopherol and 10 mg. vitamin K₃ in 5 gm. starch. This presumably was nutritionally adequate for growth in the mouse.

Diets 3,4,5 and 6 contained 35 per cent *Tricholoma* in place of all of the cellulose, all of the vitamin-free casein and part of the starch. Calculated comparisons of the amino acid contribution of diets containing 35 per cent mycelium (16 per cent protein in the diets) with the requirements for rat growth (8), indicated that methionine and phenylalanine would be limiting amino acids. *Diets 3,4,5 and 6* were designed to test the validity of these calculations. *Diet 7* involved the further addition of vitamin-free casein to investigate the possibility of amino acid deficiencies other than the two mentioned. *Diet 8* was included to determine if a higher level (50 versus 35 per cent) of mycelium would compensate for a possible low biological value of the protein and also to test further the possibility of toxicity.

¹ Solka Roc B.W.—100. Brown Corp., Montreal, Que.

² Bone meal 280, CaCO₃ 470, NaCl 250, FeSO₄ 20, MgSO₄ 0.5, CuSO₄ 0.5 and KI-calcium stearate 0.1 gm.

Diet 9 was similar to *Diet 2* except that 5 per cent *Tricholoma* was substituted for the vitamin mixture. The fat-soluble vitamins A, D, E and K were added separately; hence this diet was used for a gross evaluation of the B-complex content.

Diet 10 was similar to *Diet 7*, except that no amino acid supplements were added; hence it permitted a comparison of vitamin-free casein with a combination of methionine and phenylalanine sufficient to meet rat requirements for these amino acids.

Experiment No. 2

This test involved eight rations similar in formulation to synthetic *Diet 2* in the previous experiment, except that Drackett Assay Protein C-17¹ was substituted for vitamin-free casein. *Diets 11* to *14* inclusive contained 5 per cent of rancid corn oil, and *Diets 15* to *18* had fresh corn oil added. *Diets 11* and *15* were unsupplemented. *Diets 12* and *16* contained 0.05 per cent DPPD² (N,N'-diphenyl-p-phenylene diamine) mixed in the oil; *Diets 13* and *17* contained 10 per cent *Tricholoma* replacing 5 per cent each of starch and the vitamin mixture. *Diets 14* and *18* contained both mycelium and DPPD.

Diets 19 to *33* contained either 5 per cent fresh corn oil, 5 per cent rancid corn oil, or 5 per cent "Crisco" as the fat component. To obtain further information on the nature of the factor affecting peroxide values each of the fat treatments was further subdivided into five treatments: (a) control; (b) 10 per cent mycelium; (c) 10 per cent Skelly F solvent-

TABLE 1.—CHEMICAL COMPOSITION OF DRIED *Tricholoma nudum*

Component	Concentration
Crude protein (Nx6.25), %	44 to 50
Crude fat, %	4
Ash, %	6
Moisture, %	2
Carbohydrate, as glucose, %	24
Lysine, mg./gm.	30
Threonine, mg./gm.	17
Arginine, mg./gm.	21
Histidine, mg./mg.	13
Valine, mg./gm.	18
Methionine, mg./gm.	8
Leucine, mg./gm.	30
Isoleucine, mg./gm.	14
Phenylalanine, mg./gm.	17
Tryptophane, mg./gm.	16
Riboflavin, mg./lb.	24
Niacin, mg./lb.	68
Pantothenic acid, total, mg./lb.	66
Pantothenic acid, free, mg./lb.	54
Choline, gm./lb.	5.4
Thiamin, mg./lb.	5

¹ Drackett Products Co., Cincinnati, Ohio.

² Merck & Co., Ltd., Montreal, Que.

extracted mycelium; (d) 10 per cent of mycelium autoclaved 30 minutes at 15 lb. pressure, and (e) 10 per cent of mycelium heated at 105° C. for 24 hours. No B-vitamins were added to diets containing *Tricholoma*.

Feeding and management procedures were similar to those in Experiment 1, except that male mice were used.

RESULTS AND DISCUSSION

Chemical Composition

The chemical composition relative to the major constituents, amino acids and B-vitamins is given in Table 1. As mentioned previously, a comparison of the amino acid distribution in this material with the growth requirements of the rat indicated methionine and phenylalanine as the first and possibly the only limiting acids. It is perhaps significant that no essential amino acids were grossly deficient and none was present in great excess. However, there was a surplus of both arginine and tryptophane, relative to rat requirements, when the mycelium supplied the entire protein at a 16 per cent protein level in the diet.

The protein content of dried *Tricholoma* was in the range of 44 to 50 per cent. The fat and ash contents were typical of many common feedstuffs but it will be noted that approximately one-fifth of the material was unaccounted for in the analysis. According to Foster (3) materials of this kind have been reported to contain appreciable quantities of pentosans, hemicellulose, "cellulose" and "lignin" as well as glycerol, none of which was investigated here.

Among the vitamins assayed, all except niacin proved to be higher than the amounts contained in average quality *Torula* yeast (2).

TABLE 2.—DIETS USED IN EXPERIMENT 1 FOR EVALUATING THE AMINO ACID AND GENERAL B-VITAMIN CONTENT OF *Tricholoma*, WITH FEEDING TRIAL RESULTS

Diet No.	Description	Gains ¹	Feed intakes
		gm.	gm.
1	Stock colony diet. Positive control	9.5	54
2	Synthetic (semi-purified) basal diet	—	—
	<i>Synthetic basal containing no protein, no vitamins</i>		
3	Basal + 35% <i>Tricholoma</i> + vitamins	8.5	44
4	Basal + 35% <i>Tricholoma</i> + vitamins + 0.13% 1-methionine	9.7	45
5	Basal + 35% <i>Tricholoma</i> + vitamins		
	+ 0.30% 1-phenylalanine	8.1	44
6	Basal + 35% <i>Tricholoma</i> + vitamins		
	+ 0.13% 1-methionine	10.6	46
	+ 0.30% 1-phenylalanine	11.2	45
7	As in diet 6 + 4% vitamin-free casein	10.2	50
8	Basal + 50% <i>Tricholoma</i> + vitamins	10.7	44
9	Basal + 20% vitamin-free casein + 5% <i>Tricholoma</i>		
10	Basal + 35% <i>Tricholoma</i> + 4% vitamin-free casein + vitamins	10.4	45

¹ Necessary difference is 0.6 gm. (P=.05).

Experiment No. 1

The results of this trial are shown in Table 2. The basal diet (No. 2) proved to be deficient in potassium; however it was shown (1) that 5 to 10 per cent of *Tricholoma* added to the diet would provide the necessary potassium.

With respect to the amino acids, the inclusion of *Tricholoma* as the only source of protein and with a 16 per cent level of protein did not permit normal growth as compared to the stock diet. The addition of methionine brought growth rates to normal, whereas phenylalanine alone failed to do so. Thus methionine is the first limiting amino acid. A combination of the two amino acids resulted in a significant improvement over methionine alone and also over the stock diet, which, of course, may have resulted in part from the relatively high digestible energy content of the synthetic diet. The feeding trials thus confirmed the calculations based on amino acid analysis in relation to rat requirements.

TABLE 3.—THE EFFECTS OF *Tricholoma* PREPARATIONS AND OF DPPD ON RANCIDITY IN DIETS HELD AT 25° C. FOR 3 WEEKS

Diet No.	Ration description	Mouse gains	Peroxide values		Final
			Initial	Mid-test	
	<i>Synthetic basal plus:</i>	gm.		(m.e./kgm. fat)	
11	5% rancid corn oil	—			551
12	5% rancid corn oil + 0.05% DPPD	—			151
13	5% rancid corn oil + <i>Tricholoma</i>	11.5			28
14	5% rancid corn oil + <i>Tricholoma</i> + DPPD	13.1			16
15	5% fresh corn oil	—			604
16	5% fresh corn oil + 0.05% DPPD	—			49
17	5% fresh corn oil + <i>Tricholoma</i>	12.2			9
18	5% fresh corn oil + <i>Tricholoma</i> + DPPD	11.7			6
19	5% fresh corn oil	—	247	265	90
20	5% fresh corn oil + <i>Tricholoma</i>	12.9	6	7	8
21	5% fresh corn oil + extr. <i>Tricholoma</i>	10.1	9	7	6
22	5% fresh corn oil + autoclaved <i>Tricholoma</i>	4.4	6	5	6
23	5% fresh corn oil + heated <i>Tricholoma</i>	10.2	5	5	7
24	5% rancid corn oil	—	441	227	108
25	5% rancid corn oil + <i>Tricholoma</i>	16.2	35	17	20
26	5% rancid corn oil + extr. <i>Tricholoma</i>	10.1	37	31	40
27	5% rancid corn oil + autoclaved <i>Tricholoma</i>	4.7	17	19	19
28	5% rancid corn oil + heated <i>Tricholoma</i>	10.1	7	28	27
29	5% Crisco	—	13	82	104
30	5% Crisco + <i>Tricholoma</i>	12.2	3	4	5
31	5% Crisco + Extr. <i>Tricholoma</i>	11.7	5	7	8
32	5% Crisco + autoclaved <i>Tricholoma</i>	5.0	8	5	4
33	5% Crisco + heated <i>Tricholoma</i>	11.5	2	3	5

The addition of 4 per cent vitamin-free casein to the amino acid-supplemented diets resulted in further increases in growth and feed efficiency. This finding was not explored further to determine its nutritional significance, but the results from feeding 50 per cent of *Tricholoma* to increase the protein intake and to provide about as much of the two limiting amino acids as required indicates that protein level *per se* was not too low. It is thus possible that, if higher levels of one or both of the supplementary amino acids had been fed, growth rates would have been greater.

The results from *Diet 9* indicate that the use of 5 per cent of *Tricholoma* in place of the synthetic B-vitamin mixture allowed normal growth. This confirms and extends the conclusions based on vitamin assays to the effect that *Tricholoma* is an excellent source of B-vitamins.

Experiment No. 2

The results of this test are shown in Table 3. The gains obtained in the growth studies are not shown for those diets which were deficient in potassium (*Diets 11, 12, 15, 16, 19, 24, 29*), but it may be stated that normal growth was obtained in later work in which similar diets contained at least 0.2 per cent potassium (1). A marked depression in gains was evident with *Diets 22, 27* and *32* which contained autoclaved *Tricholoma*. Supplementary thiamin was shown later to restore the ability of these diets to support normal growth. The same applied to the dry-heated *Tricholoma* treatments but it is not evident what dietary factor was affected by solvent extraction.

Of most interest in the experiment was the finding that *Tricholoma* treated or untreated, was associated with low peroxide values. The peroxide titres were 50 and 4 m.e./Kgm. fat for rancid and fresh corn oils, respectively, before they were incorporated in the rations. The values for diets containing fresh oil remained near the initial value over the 21-day period in the presence of the mycelium but showed marked rancidity in its absence. There was a suggestion of an additive effect between *Tricholoma* and DPPD (*Diets 13, 14, 17, 18*).

The peroxide values in diets to which rancid oil had been added were consistently lower than the initial values and the diets remained relatively free of the rancid odours characteristic of the control rations. It is noted that the peroxide data are not related to the growth rates of the mice; hence the treatments of the *Tricholoma* had no obvious effect upon the peroxide responses.

No explanation can be offered at this time for the apparent effects of *Tricholoma* on peroxide values but the finding is of special interest with regard to the incorporation and utilization of fats in feeds. Beyond this it is noteworthy that *Tricholoma nudum* residue was found to be approximately equal to *Torula* yeast in B-vitamin content and to be a relatively good source of arginine and tryptophane. The first limiting amino acid was methionine.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the assistance of H. D. Branion and E. V. Evans, Department of Nutrition, Ontario Agricultural College, Guelph, Ontario, who provided the vitamin data, and of B. M. Craig, Prairie Regional Laboratory, who determined the peroxide values.

REFERENCES

1. Bell, J. M., and J. D. Erfle. The requirement for potassium in the diet of the growing mouse. *Can. J. Animal Sci.* 38: 144-146. 1958.
2. Committee on Feed Composition. Composition of concentrate by-product feeding-stuffs. Publ. 449., N.A.S., National Research Council, Washington, D. C. 1956.
3. Foster, J. W. Chemical activities of fungi. Academic Press, New York, N.Y. 1949.
4. Mathias, W. Serienuntersuchungen mit Hilfe neuem Form der Streifen-Papier-chromatographie. *Naturwissenschaften* 41:17-18. 1954.
5. Neish, A. C. Analytical methods for bacterial fermentations, N.R.C. 2952. Prairie Regional Laboratory, Saskatoon, Sask. 1952.
6. Reusser, F., J. F. T. Spencer, and H. R. Sallans. *Tricholoma nudum* as a source of microbiological protein. *Applied Microbiol.* 6: 5-8. 1958.
7. Reusser, F., J. F. T. Spencer, and H. R. Sallans. Essential amino acids in microorganisms. *Can. J. Microbiol.* 3: 721-728. 1957.
8. Rose, W. C. The nutritive significance of the amino acids. *Physiol. Revs.* 18:109-136. 1938.
9. Sully, B. D. A modified iodimetric determination of organic peroxides. *Analyst* 79:86-90. 1954.

FLAX HAY AND STRAW AS FEEDS FOR SHEEP¹

F. WHITING

Canada Department of Agriculture, Lethbridge, Alberta

[Received for publication September 17, 1958]

ABSTRACT

Feeding and digestion experiments were carried out with range sheep on flax hay, timothy-alfalfa hay, flax straw, and oat straw. The flax hay and the timothy-alfalfa hay were similar in chemical composition, digestibility, and feeding value. Both hays contained approximately 6.4 per cent D.C.P. and 53 per cent T.D.N.

The flax straw and the oat straw were unpalatable to sheep. Flax straw was lower in digestible protein and energy than the oat straw. The D.C.P. and T.D.N. contents were 0.1 and 39, and 1.8 and 51 per cent for flax and oat straws, respectively.

It is concluded that flax cut when the plants are immature makes a hay similar in feed value to a hay composed of equal parts of timothy and alfalfa, providing the Prussic acid content is not above toxic levels. Mature flax straw is inferior in feed value to oat straw of the same maturity.

INTRODUCTION

Flax is not normally grown for a hay or feed crop in Canada. However, during some years part of the crop is damaged by frost before it reaches maturity and can be salvaged only as feed for live stock. Also, each year there are many tons of flax straw that could be used as feed. There is very little information available on the value of flax straw or immature flax preserved as hay as feeds for live stock. It is known that frost damage and other factors may result in toxic levels of Prussic acid (HCN), especially in immature flax plants (6).

Wilson *et al.* (7) found that flax straw (quality or chemical composition not given) had a high feed value when fed as the only roughage or with alfalfa hay, plus a small allowance of corn to pregnant cows and yearling steers. Ince (3) reported that, based on farmers' experiences, flax straw had considerable value as a feed and was quite palatable. He reported that flax straw contained approximately 9 per cent protein, 4 per cent ether extract, and 4 per cent ash. Morrison (5) lists flax straw as containing 6 per cent digestible crude protein (D.C.P.) and 38 per cent total digestible nutrients (T.D.N.).

Fatal poisoning of sheep (100 lb.) has been reported to occur when they have consumed as little as 0.5 lb. (fresh weight) of arrow grass containing 0.05 per cent HCN. Ince (4) found that the HCN content of samples reported to have caused death among cattle and sheep has varied from 0.057 to 0.243 per cent. Couch (2) found that, if a cow or horse rapidly consumed as much as 5 lb., and sheep 1.25 lb. of a plant containing 0.02 per cent potential HCN, it was fatal if no other factors prevented the development of the acid. Apparently many factors, such as the rate of eating and the presence of other feeds in the stomach, will modify the amount of HCN formed (6).

¹ Contribution from the Division of Animal Husbandry, Experimental Farms Service, Canada Department of Agriculture, Ottawa, Ont.

PROCEDURE

Flax Hay

Thirty-four range wethers (approximately 12 months old) were divided into two equal lots on the basis of body weight and fed the following rations:

Lot 1—flax hay

Lot 2—mixed timothy-alfalfa hay.

The flax hay was from a crop grown during 1956 at Claresholm, Alberta, and which had been damaged by frost when still green. (The seed bolls had formed but were still very immature). The crop was swathed within a week after the frost damage, field-cured, baled, and stored until the summer of 1957. The crop contained 0.062 per cent HCN when cut for hay, and 0.036 per cent when fed the following summer*. The timothy-alfalfa hay was a mixture of equal parts by weight of timothy and alfalfa hay. Both hays were medium to good quality.

Both lots of sheep were fed the same daily amount of hay, the amount fed being based on that consumed by the lot with the poorer appetite. No concentrate was fed. Salt and water was available at all times.

The experiment was of 12 weeks' duration. The sheep were weighed individually at 2-week intervals.

The digestibility of the flax and the timothy-alfalfa hay was determined, using 6 yearling wethers for each hay. Each digestion trial consisted of a 10-day preliminary period followed by a 10-day collection period.

Official methods of the A.O.A.C. (1) were used in the chemical analyses of all feed and feces samples.

Flax Straw

Thirty-two ewe lambs, approximately 7 months old, were divided into two equal lots and fed the following rations:

Lot 3—flax straw plus grain

Lot 4—oat straw plus grain.

The flax straw was field-baled after the crop had been harvested with a combine. The crop was mature when harvested and relatively free from weeds. The oat straw was from a crop that was harvested when mature with a binder and later threshed. It was good quality straw but contained very little chaff.

One-half pound of grain (equal weights of rye and oats) was fed per lamb daily during the first 4 weeks on feed, one pound daily during the next 6 weeks, and one-half pound each of oats and linseed oilmeal daily during the last 4 weeks on feed.

Roughage was hand-fed daily in amounts that the lambs would consume without much waste. The lambs were weighed individually at 2-week intervals.

The digestibility of the flax and oat straw was determined as described above for flax hay.

* The author acknowledges the assistance of J. L. Doughty and W. C. Hinman, Experimental Farm, Swift Current, in determining the HCN content of the flax hay.

RESULTS

Flax Hay

The chemical composition and digestibility of the flax and the timothy-alfalfa hays are shown in Table 1, and the gains and feed consumption of the sheep when fed these hays are shown in Table 2. The flax hay and the timothy-alfalfa hay were very similar in chemical composition and digestibility, the only significant difference being in the amount and digestibility of ether extract.

The flax hay was more palatable to the sheep than the timothy-alfalfa hay, as judged by the length of time it took the sheep in each lot to consume their daily allowance of hay. There was no difference ($p < 0.05$) between the body weight gains of the sheep fed flax hay and those fed the timothy-alfalfa hay.

The high HCN content (0.036 per cent) of the hay when fed did not have any noticeably harmful effects on the sheep in this experiment. It is generally considered that hay containing more than approximately 0.02 per cent HCN is potentially harmful.

These results would indicate that green flax preserved as hay is comparable in feeding value to a grass-legume hay of similar quality. Before feeding flax hay, its HCN content should be checked.

TABLE 1.—THE AVERAGE PERCENTAGE CHEMICAL COMPOSITION AND DIGESTIBILITY BY SHEEP OF FLAX HAY, TIMOTHY-ALFALFA HAY, FLAX STRAW, AND OAT STRAW

	Flax hay	Timothy-alfalfa hay	Flax straw	Oat straw
Chemical Composition				
Dry matter	90.1	90.4	90.4	89.3
Protein (N \times 6.25)	10.1	10.6	2.4	4.3
Crude fibre	34.3	35.0	56.9	36.0
Ether extract	4.3	1.6	1.4	1.9
Ash	5.9	6.3	2.7	6.3
Nitrogen-free extract	35.5	36.9	27.0	40.8
Gross energy (Cal./gm.)	4.1	3.9	3.8	3.9
Calcium	0.75	0.85	0.51	0.57
Phosphorus	0.16	0.15	0.02	0.08
Digestion Coefficients				
Dry matter	57	61	44	62
Organic matter	58	62	44	60
Protein (apparent)	62	61	6	43
Crude fibre	50	52	48	60
Ether extract	79	45	36	61
Nitrogen-free-extract	62	60	38	63
Gross energy	58	60	44	58
D.C.P.	6.3	6.5	0.1	1.8
T.D.N.	53	53	39	51
Dig. energy (Therms/100 lb.)	110	108	76	103

TABLE 2.—BODY WEIGHT GAINS AND FEED CONSUMPTION OF SHEEP FED FLAX HAY, TIMOTHY-ALFALFA HAY, FLAX STRAW, AND OAT STRAW

	Lot 1	Lot 2	Lot 3	Lot 4
	Flax hay	Legume-grass hay	Flax straw	Oat straw
Number animals per lot	17	17	16	16
No. weeks on feed	12	12	14	14
Av. initial weight (lb.)	77	76	91	91
Av. final weight (lb.)	90	90	93	95
Av. gain (lb.)	13	14	2	4
Av. daily feed consumption (lb.)				
Roughage	3.3	3.3	1.0	1.4
Grain ¹	—	—	0.9	0.9

¹ Includes the linseed oilmeal fed.

Flax Straw

The chemical composition and digestibility of the flax and oat straws are shown in Table 1 and the gains and feed consumption of the sheep when fed these straws are shown in Table 2. In comparison with oat straw, the flax straw contained more fibre and less protein and total digestible nutrients. The digestibility of the oat straw was higher, and that of the flax straw was lower than that reported by other investigators (3, 5). The reason for this is not known.

Both the flax and oat straws were unpalatable to the sheep. During the first 4 weeks, when one-half pound of grain was fed, both lots lost weight (approximately 1 lb. per lamb). When one pound of grain was fed daily during the next 6 weeks both lots gained an average of 2 lb. per lamb and when one-half pound of grain and one-half pound of linseed oilmeal was fed daily during the following 4 weeks, the lambs fed flax straw gained an average of 1 lb. and those fed oat straw gained an average of 3 lb. These data would indicate that available energy was the factor limiting body gains in both lots. Flax straw of a quality similar to that used in this experiment would have a very low feed value for any class or age of live stock.

REFERENCES

1. Beath, O. A., C. S. Gilbert, H. F. Eppson, and Irene Rosenfeld. Poisonous plants and livestock poisoning. Wyoming Agr. Expt. Sta. Bull. 324. 1953.
2. Couch, James F. Poisoning of livestock by plants that produce hydrocyanic acid. U.S. Dept. Agr. Leaflet 88. 1934.
3. Ince, J. W. An investigation of the feeding value of flax material as determined by chemical analysis. N. Dakota Agr. Expt. Sta. Bull. 106:10-29. 1913.
4. Ince, J. W. A further study on the distribution of Prussic acid in the flax plant. N. Dakota Agr. Expt. Sta. Bull. 106:30-46. 1913.
5. Morrison, Frank B. Feeds and feeding. 22nd ed. Morrison Publ. Co., Ithaca, N.Y. 1956.
6. Udall, D. H. The practice of veterinary medicine. Publ. by author, Ithaca, N.Y. 1947.
7. Wilson, James W., Turner Wright, and Forrest Fenn. Feeding flax straw to cattle. S. Dakota Agr. Expt. Sta. Circ. 3. 1932.

PASTURE MANAGEMENT STUDIES.

I. DAILY STRIP GRAZING VERSUS FREE RANGE GRAZING OF DAIRY CATTLE ON CULTIVATED PASTURE¹

V. S. LOGAN AND VERN MILES

Canada Department of Agriculture, Ottawa, Ontario

[Received for publication August 11, 1958]

ABSTRACT

Daily strip grazing was compared with free range grazing for dairy cows during three pasture seasons on replicated areas in a 5-year crop rotation. Seventy-two Holstein and Ayrshire cows in early lactation and twelve yearling heifers were used for the complete experiment.

Pasture productivity was estimated by crediting the respective pasture plots with the T.D.N. required for the milk produced, for maintenance and gain, and for the estimated T.D.N. harvested as silage or hay. The T.D.N. fed in supplementary feed (silage, hay, and concentrate) and the T.D.N. equivalent of any body weight losses were deducted from the pasture production.

Strip-grazing yields were 16.5 to 26.9 per cent higher than those from free range grazing. Surplus grass cut and supplement adjustment accounted for the major portion of these differences. While strip grazing appeared to maintain more persistent milk yields, total milk production did not differ significantly between the two systems. Milk composition (butterfat and solids-not-fat) did not differ significantly between the two systems of grazing and was not affected by the varying stages of pasture growth during strip-grazing cycles.

INTRODUCTION

Intensive rotational grazing has been effective in increasing the carrying capacity of pastures (3, 10). In general, pasture productivity increases through more intensive grazing with a larger number of sub-divisions of the pasture. Strip grazing (also termed "ration" grazing) was tried experimentally in Holland in 1946 by de Geus (4). Cows were restricted by electric fences to an area of pasture sufficient for one day's requirements. Using this system de Geus increased pasture yields, in terms of starch equivalent, by 24 per cent over rotational grazing. Holmes *et al.* (5, 6) and Procter *et al.* (11) reported increased pasture productivity ranging to 45 per cent for strip grazing over rotational grazing. The practice has been extensively tested in New Zealand (8). Brundage and Peterson (2) reported pasture yields per acre from daily strip grazing to be nearly three times that of continuous grazed pasture.

In Eastern Canada, rapid growth of pasture grass during May and June may account for 65 per cent or more of the season's pasture (7). A period of retarded growth follows in late July and through August. If pastures are free range grazed, supplemental feed is frequently required during this period. At the same time considerable waste occurs through loss of nutrients when excess spring growth becomes dry, unpalatable, and of lower feeding value. Ordinary rotational grazing as a means for more efficient utilization of pasture has not been adopted widely in Eastern Canada, probably due to the extra fencing and watering facilities required.

¹ Contribution from the Division of Animal and Poultry Science, Experimental Farms Service, Ottawa, Ont.

An experiment was begun in 1952 at the Experimental Farm, Ottawa, to determine the relative pasture productivity when restricting cows to daily grazing areas as compared to free range grazing; also to measure any influence on performance of the cows, including variation in milk composition under the two systems of grazing. A brucellosis outbreak in 1952 necessitated the replacement of a number of the experimental animals. The results for the first year, therefore, were not considered sufficiently accurate for inclusion with those for the succeeding 3 years reported here.

MATERIALS AND METHODS

Pasture Area

The pasture used each season followed hay in a 5-year crop rotation. The swards contained alfalfa, red clover, alsike, timothy, and orchard grass, in which the predominant species were alfalfa and orchard grass.

Replicated comparisons of the two grazing systems were made each year by dividing a 24-acre area into four equal fields, except for the season of 1954 when a 20-acre area was subdivided. The areas used were slightly in excess of anticipated grazing requirements, thus permitting the initial allotment of animals to be maintained throughout each season. Surplus grass during lush spring growth was harvested as silage or hay, and yields recorded. The T.D.N. content was determined from proximate principle analyses of samples, using Morrison's (9) digestion coefficients.

All pasture fields were closed out in a uniform condition of forage growth at the end of each season.

TABLE 1.—DESCRIPTION OF ANIMALS ALLOTTED TO PASTURE TREATMENT

Year	Group no.	Pasture treatment	Animal numbers	Initial av. wt.	Initial av. daily prod.	Av. age	Av. time since freshening
				lb.	lb.	yr.	days
1953	1	Strip	5 cows	1208	34.5	4.2	95
			3 heifers	873	—		
	2	Free Range	5 cows	1239	35.5	3.6	89
			3 heifers	876	—		
	3	Strip	5 cows	1202	36.5	3.7	88
			3 heifers	874	—		
	4	Free Range	5 cows	1206	35.0	3.7	101
			3 heifers	889	—		
1954	1	Strip	6 cows	1111	38.4	3.7	47
	2	Free Range	6 cows	1096	39.1	4.5	50
	3	Free Range	6 cows	1163	38.9	4.2	49
	4	Strip	6 cows	1156	39.5	4.5	48
1955	1	Free Range	7 cows	1202	37.3	4.7	92
	2	Strip	7 cows	1180	36.9	3.9	92
	3	Free Range	7 cows	1215	36.9	4.6	94
	4	Strip	7 cows	1230	36.4	4.7	92

Animals

Holstein and Ayrshire cows in early lactation were allotted into four uniform groups as to breed, stage of lactation, body weight, and daily milk yields. For 1953, equal numbers of yearling heifers of uniform weight were used to complete the required numbers in each group. During the season of 1955, two cows became infected with mastitis and were replaced with comparable animals. The composition of the groups a week before the beginning of each season is shown in Table 1.

Grazing Management

Cows on the free range grazed fields were limited by a temporary electric fence to two-thirds of the allotted areas at the beginning of each season. After one cutting of grass was removed from the fenced-off areas, the aftermath was made accessible to the cows for the remainder of the season.

On the strip grazed fields the forward fence was strung between the fences dividing the fields and advanced each morning after milking to provide a fresh strip of grass. The distance the fence was moved each day was estimated from a combination of (a) the extent the area provided the previous day was grazed, and (b) how well milk production was being maintained. A second fence was strung behind the cows to protect aftermath growth on the grazed area and provide an extending lane to the gate entrance for direct access to the day's grazing. The rear fence was moved at weekly intervals and placed to allow access to the previous day's grazed area in addition to the day's grass allowance to reduce contamination of the ungrazed grass by droppings.

This grazing procedure was continued along the field until aftermath on the initially grazed sward had recovered sufficiently for regrazing. The cows were then moved back and the cycle repeated. Surplus grass from the strip grazed fields was cut and ensiled on completion of the first cycle of grazing. At the same time the reserved portion of the free range grazed fields was cut and ensiled. At the end of the second cycle of strip grazing, surplus forage was harvested as hay. During each season the third cycle of grazing extended over the entire field before the initially grazed aftermath had recovered sufficiently. The rear fence was removed during the fourth cycle of strip grazing since aftermath recovery in late season was negligible.

Supplementation

A simple meal mixture of oats and barley was fed twice daily to all cows in accordance with rate of production. The cows had ready access to mineral and water supplies.

Silage cured from surplus grass was fed back as pastures became depleted. All silage fed was recorded as supplement against the respective treatments.

Recording and Sampling

All cows were weighed at 10 a.m. on two consecutive days at the beginning and close of each pasture season. Single weights were taken

at 14-day intervals. The cows were milked twice daily and the recorded yields were converted to 4 per cent fat-corrected-milk (F.C.M.) to determine T.D.N. required for production. Forage harvested and supplemental feeds fed were routinely sampled for proximate principle analyses.

Pasture productivity was determined by the method outlined by Sylvestre and Williams (13) through reverse application of feeding standard requirements. Thus, pasture productivity expressed as T.D.N. was calculated from the total of the T.D.N. requirements for milk production, maintenance, body weight gain, plus T.D.N. in harvested forage. The T.D.N. in meal and silage supplements and that determined for body weight loss were deducted.

Milk samples taken at 2-week intervals from each cow during the 1955 season were analysed by the Majonnier method for fat and solids-not-fat content.

The data for milk yields, body weight change, and milk composition were tested by the analysis of variance method (12).

RESULTS AND DISCUSSION

Pasture and Animal Production

A summary of the comparisons between the two grazing systems is given in Table 2. The replicated comparisons were consolidated in each season's results.

TABLE 2.—SUMMARY OF YIELDS UNDER STRIP VERSUS FREE RANGE GRAZING BY SEASONS

	1953		1954		1955	
	Strip grazed	Free range	Strip grazed	Free range	Strip grazed	Free range
Days on pasture	140	140	150	150	150	150
Av. daily gain per head (cows)	lb. 0.51	0.34	0.20	0.16	0.24	0.10
Av. 4% F.C.M. per head per day	lb. 30.5	28.5	32.9	32.9	30.6	31.1
T.D.N. produced per acre	lb. 3664	2984	4288	3378	3486	2991
Increased yield of strip over free range grazing	% 22.8		26.9		16.5	
Carrying capacity 150-day season, A.U./ac./day*	1.53	1.24	1.79	1.41	1.45	1.25

* Animal Unit per acre, per day = 16 lb. T.D.N.

Differences in body gain were not significant though Table 2 shows that the average daily body gains each season were slightly higher for cows on strip grazing than for those on free range. Analysis of variance of fat-corrected milk yields showed no significant between-treatment differences ($P=0.05$). T.D.N. yields were consistently higher for the strip grazed than for the free range grazed fields.

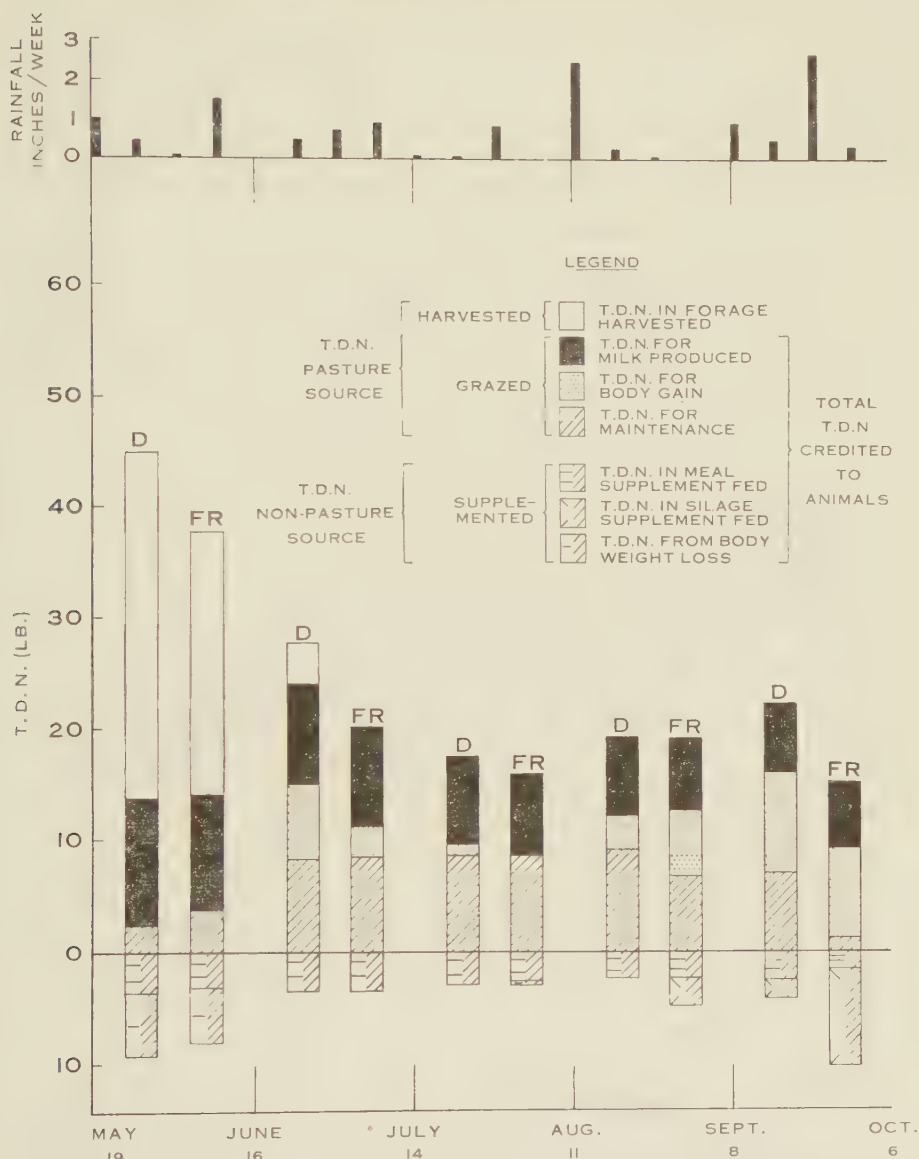


FIGURE 1. 1953 Comparison of pasture productivity, animal performance, and supplementation on daily strip (D) versus free range grazing (FR). Yields are shown by periods on a per acre/day basis in conjunction with rainfall.

At the beginning of each pasture season the cows on strip grazing were pastured at a stocking rate of approximately 60 cows per acre per day. As the rate of grass growth increased the daily areas were reduced, so that stocking rate increased to approximately 120 cows per acre per day. Grazing intensity was reduced to less than one-quarter of the peak rate as grass recovery became retarded after mid-season. While the animals

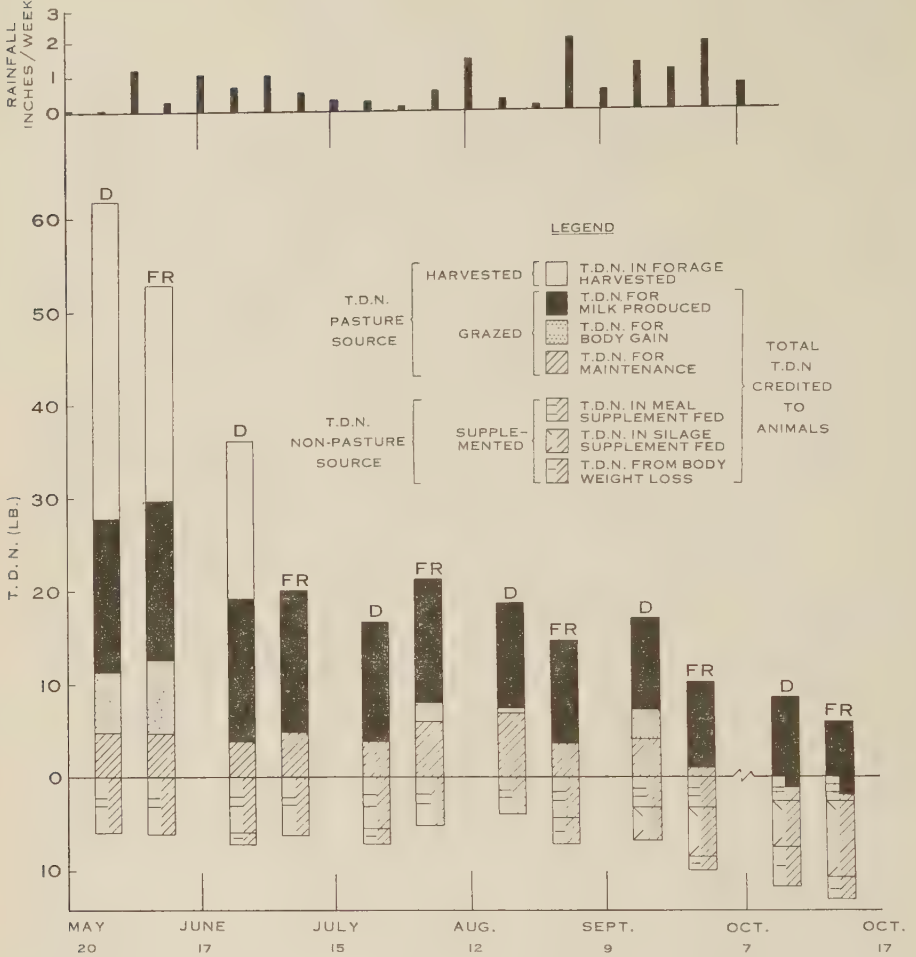


FIGURE 2. 1954 Comparison of pasture productivity, animal performance, and supplementation on daily strip (D) versus free range grazing (FR). Yields are shown by periods on a per acre/day basis in conjunction with rainfall.

had a tendency to sort and graze the grass species first, and the legumes later, each daily area was closely grazed during the initial season cycles. During subsequent grazing cycles the cows delayed or refused to graze the rank grass caused by droppings during previous grazing. Toward the end of the initial grazing cycles the cows were inclined to consume the leafy material and leave the stems when forage had reached an advanced stage of growth.

The cows tended to select and overgraze certain areas on the free range fields while on the remaining areas the quality of the forage deteriorated with maturity. Refusal of rank grass caused by droppings was more pronounced on the free range fields. The cows overgrazed the fresh aftermath for a short period when allotted the remainder of the areas designated for the free range treatments each season.

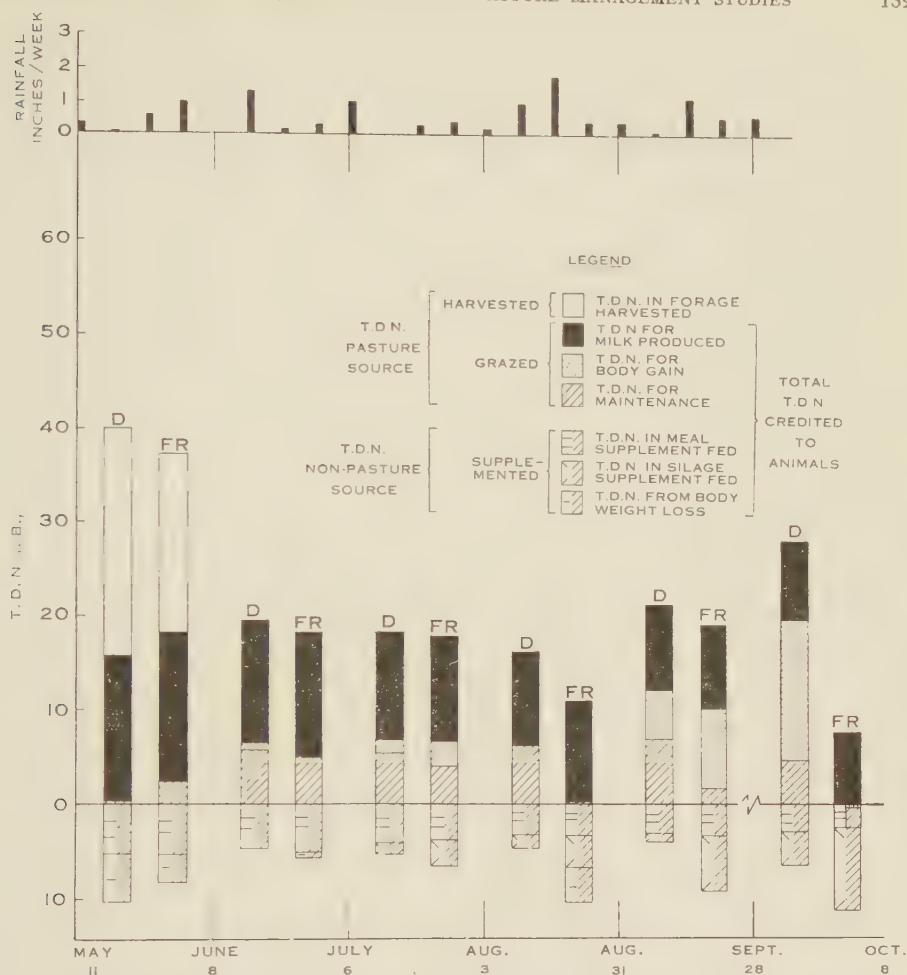


FIGURE 3. 1955 Comparison of pasture productivity, animal performance, and supplementation on daily strip (D) versus free range grazing (FR). Yields are shown by periods on a per acre/day basis in conjunction with rainfall.

Figures 1, 2, and 3 compare graphically the pasture yields in conjunction with the proportion of supplementary feeds fed under the two systems of pasture management. The bars of each graph representing the T.D.N. utilized by the animals for maintenance, body gain, and milk production, also that in the surplus forage harvested, straddle the zero line, to indicate the T.D.N. obtained from pasture above the zero line and that of non-pasture source below the zero line. Each bar is halved below the zero line, to show the proportions of T.D.N. supplied by meal and silage supplements or drawn from body reserves (weight loss). In 2 years (Figures 2 and 3) the supplements fed in late season on deficient pasture provided nutrients equivalent to the requirements for body maintenance, plus part of the requirements for milk production or body gain.

Weekly rainfall by periods is included in each figure to show seasonal variation.

Comparison of Pasture Yields by Periods

Variation in pasture yields by periods for the two management systems is shown in Figures 1, 2, and 3. During the first 28-day period of seasons 1953 (Figure 1) and 1955 (Figure 3) the cows on both grazing procedures showed appreciable net weight losses, while during the first period of 1954 (Figure 2) both showed weight increases. Owing to wet conditions in the spring of 1954 grass growth was well advanced by the time the ground was suitable for turning the cows out. This condition may have caused a more rapid weight recovery, following the initial loss due to the change from winter feeding. Balch and Line (1) found that body weight loss occurred for approximately 4 days on pasture following an abrupt change from winter feeding. They attributed this loss to variation in weight of the digesta in the reticulo-rumen and gut. While this phenomenon introduces an error in the accuracy of estimating pasture productivity at the beginning of each season, the effect on the comparison of the two grazing methods should be negligible, since all cows were fed the same before allotment to pasture treatments.

Heifers allotted to the groups in 1953 showed a greater reaction to the change from winter feeding than did the cows and accounted for two-thirds of the initial body weight loss as measured in T.D.N. For the remainder of the season the proportion of nutrients derived from pasture was in favour of the daily grazed animals. A higher rate of silage supplementation was required for the free range animals in periods 4 and 5.

For the first three periods of the 1954 season, the free range grazed animals obtained slightly more (4 per cent) of their T.D.N. requirements from pasture than did the daily grazed animals. During the remainder of the season the proportion of T.D.N. obtained from strip grazing was 16 per cent higher than from free range grazing. These results were associated with advanced grass growth when the cows were turned on pasture. On the strip grazed fields, during the second and third periods, it would appear that, in forcing the cows to clean up daily allotments of the maturing forage towards the end of the grazing cycles, insufficient areas may have been provided which could result in these groups showing a slight loss in weight. On the other hand, the free range groups, by selective grazing, maintained body weight or made slight gains. Milk production was not significantly affected by either system under these conditions.

In contrast to 1954, early spring weather in 1955 advanced the grazing by some 10 days, compared to the average date for this area. Under these conditions the difference in T.D.N. yields between the two systems for the first three periods was reduced (Figure 3). Although the free range cows were allowed the aftermath on the remainder of the 6-acre fields early in the third period, hot dry weather retarded grass growth to the extent that silage supplementation was necessary before the end of the third period. The higher rate of silage supplementation required for the free range animals during the latter part of the grazing season, accounting for a large portion of the differences in T.D.N. yields between the two systems, is evident in Figure 3.

No differences between grazing methods could be related to rainfall.

TABLE 3.—SEGREGATION OF SOURCES OF T.D.N. ACCOUNTING FOR THE INCREASED PRODUCTIVITY OF STRIP GRAZED OVER FREE RANGE GRAZED FIELDS (INCREASES EXPRESSED AS PER CENT OF SEASONAL T.D.N. PRODUCTION OF FREE RANGE)

Sources of T.D.N.	1953	1954	1955
	%	%	%
Maintenance	*-0.1	*-0.5	0.0
Gain	2.4	0.5	2.6
Milk	2.5	0.3	*-0.9
Silage supplement	8.5	2.6	9.4
Meal supplement	*-1.4	0.4	0.6
Silage, Hay—harvested	10.9	23.6	4.8
Total T.D.N.	22.8	26.9	16.5

* Minus values indicate instances where free range exceeded strip grazed.

Yearly Variation in Yield Differences

The increased productivity from strip grazed pasture over other systems of pasture management has been largely attributed to the salvaging of surplus forage and better utilization of available herbage through more intensive grazing (2). The sources of T.D.N. accounting for the increased productivity of strip grazing over free range grazing in this experiment are shown in Table 3. These have been segregated and in accordance with the management procedure followed account for the gross yield differences ranging from 16.5 to 26.9 per cent.

Milk Yields in Relation to Grazing Management

The mean daily milk yields per cow by grazing methods during the successive seasons are shown in Figure 4. The cycles of strip-grazing moves are indicated, together with the date of grazing of the free range aftermath areas. There was no indication that the change from more mature herbage to aftermath, at the beginning of each grazing cycle on the strip grazed fields, had a stimulating effect on milk yields. There was little consistency in the lactation patterns that could be directly related to management procedures, except that the daily rate of production was maintained at a higher level toward the end of the season with strip grazing.

Influence on Milk Composition

Determinations for butterfat and solids-not-fat were made on 88 milk samples (44 per treatment) during the 1955 season.

An analysis of variance of the data showed no significant differences between treatments and periods ($P=0.05$). Also, treatment \times period interaction was not significant ($P=0.05$). There were no trends in the variation of the percentage composition of the milk components that could be related to cyclical variation in composition of herbage on the strip grazed fields as differing from that of free range grazing.

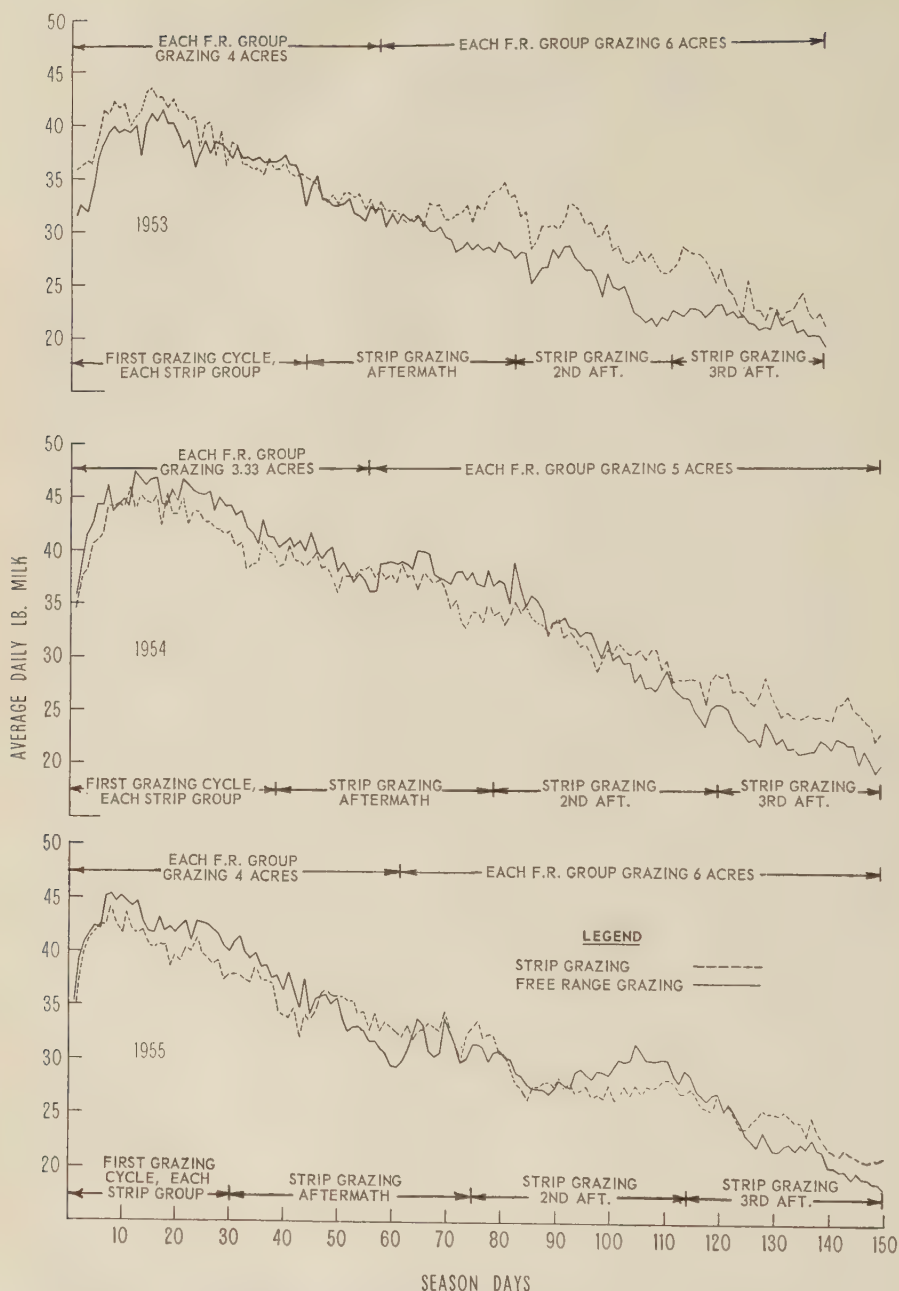


FIGURE 4. Comparison of average daily, per cow, milk yields in relation to grazing cycles and extended free range (FR) area in each of three seasons.

CONCLUSIONS

The differences in pasture productivity between the daily strip grazing system and free range grazing showed a similar trend to, although the

differences were not as great as, those found by Brundage *et al.* (2), using a similar method of evaluating T.D.N. yields from pasture. Their results, based on a single, comparatively short grazing season, showed T.D.N. yields from strip grazing to be nearly three times the production from continuously grazed pasture. The high margin of advantage for the strip grazing system reported by these workers, as differing from the three-season average of 22 per cent for this experiment, may be accounted for in part by different climatic conditions.

Under the conditions of this experiment, the proportion of surplus grass cut from the strip grazed fields and the higher rate of supplementation required to maintain production of the free range animals were the main factors contributing to the differences in pasture productivity under the two systems (Table 3). The exceptionally early grass growth in 1954 resulted in a high proportion of forage being harvested from strip grazed fields, which accounted for most of the difference between the pasture treatments that season.

The consistent advantage shown for daily strip grazing as applied to cultivated pasture through three pasturing seasons shows that dairy farmers could profitably adopt this system of pasture management. That the system is particularly adapted to efficiently utilize pasture under the conditions of varying seasonal growth in this region is clearly indicated by the high stocking rate necessary, of 120 cows per acre in early season as compared to an extreme of 80 cows per acre on good quality pasture, reported by Holmes *et al.* (5). A further consideration is that with strip grazing an adjustable reserve of grass can be made available during dry periods of the season when growth becomes retarded. An important problem with strip grazing is the accurate synchronization of harvesting operations with herd grazing requirements to avoid forced grazing of mature grass.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to members of the Analytical Chemistry Unit, Chemistry Division, Science Service, Canada Department of Agriculture, for chemical analyses of milk and feed samples; and to W. J. Pigden and K. Rasmussen for helpful suggestions in the preparation of the manuscript. J. Batty and L. Hanny, Divisional Foreman and Herdsman, respectively, provided valuable assistance in the detailed execution of this study.

REFERENCES

1. Balch, C. C., and C. Line. Weight changes in grazing cows. *J. Dairy Research* 24:11-19. 1957.
2. Brundage, A. L., and W. E. Petersen. A comparison between daily rotational grazing and continuous grazing. *J. Dairy Sci.* 35:623-630. 1952.
3. Brunton, W. Intensified grazing. *J. London Farmers' Club* 1:1-20. 1928.
4. de Geus, J. A note on ration grazing in Holland. *J. Brit. Grassland Soc.* 525:1-252. 1950.
5. Holmes, W., R. Waite, D. L. Fergusson, and Jean I. Campbell. Studies in grazing management. I. A comparison of the production obtained from close-folding and rotational grazing of dairy cows. *J. Agr. Sci.* 40:381-402. 1950.

6. Holmes, W., R. Waite, D. L. Fergusson, and D. S. MacLusky. Studies in grazing management. II. A comparison of close-folding and rotational grazing of dairy cows on intensively fertilized pasture. *J. Agr. Sci.* 42:304-313. 1952.
7. Logan, V. S. Grazing management of dairy cattle. *Agr. Inst. Rev.* 11:33-35. 1956.
8. McMeekan, C. P. Controlled grazing for increased butterfat production. *Dairy Farming Annual*, p. 116-129. 1948.
9. Morrison, F. B. Feeds and feeding. 21st ed. Morrison Pub. Co., Ithaca, N.Y. 1948.
10. Peter, F. The Hohenheim system. *J. Amer. Soc. Agron.* 21:628. 1929.
11. Procter, J., A. E. M. Hood, W. S. Fergusson, and A. H. Lewis. The close-folding of dairy cows. *J. Brit. Grassland Soc.* 5:243-250. 1950.
12. Snedecor, G. W. Statistical methods. Iowa State College Press, Ames, Iowa. 1948.
13. Sylvestre, P. E., and S. B. Williams. Methods of measuring the relative productivity of pasture experiments with livestock. Rev. mimeo. copy. Animal Husb. Div., Exptl. Farms Service, Can. Dept. Agr., Ottawa. 1952.

THE REQUIREMENT FOR POTASSIUM IN THE DIET OF THE GROWING MOUSE

J. M. BELL¹ AND J. D. ERFLE²

University of Saskatchewan, Saskatoon, Saskatchewan

[Received for publication July 28, 1958]

ABSTRACT

In the studies with Carworth Farms No. 1 weanling white mice and involving semi-purified diets supplemented with various levels of KCl, or with natural feedstuffs rich in potassium, it was found that the potassium requirement for normal growth was approximately 0.2 per cent of the diet.

INTRODUCTION

In a literature survey³ of the nutrient requirements of the mouse it was evident that no information had been published on the dietary requirement for potassium. Recent reports indicate that the growing rat needs 0.18 per cent potassium (2) and the chick 0.16 to 0.20 per cent, depending on stage of growth (3).

During an investigation of the nutritional value of the fermentation residue from *Tricholoma nudum* (1) there was occasion to employ a mineral mixture devoid of potassium in semi-purified diets that were adequate nutritionally in other respects. Since several levels of potassium (as KCl) and various natural feedstuffs were used, it was possible to establish an estimate of the potassium requirement for the growing mouse.

MATERIALS AND METHODS

Basal Diets

The basal diet was composed of corn starch 44.7; sucrose 11.2; cellulose 11.2; mineral mixture 5.6; vitamin-free casein 22.3, and vitamin mixture 5.0 per cent. The mineral mixture contained bone meal 26.9; ground limestone 45.1; NaCl 24.0, FeSO₄·7H₂O 1.9; MgSO₄·7H₂O 1.9, MnSO₄·5H₂O 0.05, CuSO₄·5H₂O 0.05, and KI-calcium stearate 0.01 per cent. The vitamin mixture, carried in 5 gm. of corn starch, was made up of 0.30 mg. thiamin; 0.40 mg. riboflavin; 0.30 mg. niacin; 0.01 mg. biotin; 0.10 mg. pyridoxine; 0.50 µg. vitamin B₁₂; 2.5 µg. folacin, 0.90 mg. pantothenic acid; 0.10 mg. inositol; 135 mg. choline chloride; 1.25 mg. para-amino-benzoic acid; 0.15 mg. Vitamin A (325,000 I.U./gm.); 5 µg. vitamin D (37,383,000 I.U./gm.); 11 mg. vitamin E (125,000 I.U./lb.) and 10 mg. vitamin K per 100 gm. of diet.

Animals

Four male weanling mice of Carworth Farms No. 1 strain were randomly allotted to each of the diets tested. The mice weighed 8.0 to 9.0 gm. initially and did not exceed 23 days of age. During the study the mice were housed individually in wire-bottom, metal cages in a mobile

¹ Professor and Head, Department of Animal Husbandry, University of Saskatchewan, Saskatoon, Sask.

² Graduate Assistant, Department of Animal Husbandry, University of Saskatchewan, Saskatoon, Sask.

³ Conducted on behalf of the Subcommittee on Laboratory Animals, National Research Council, National Academy of Sciences, Washington, D.C.

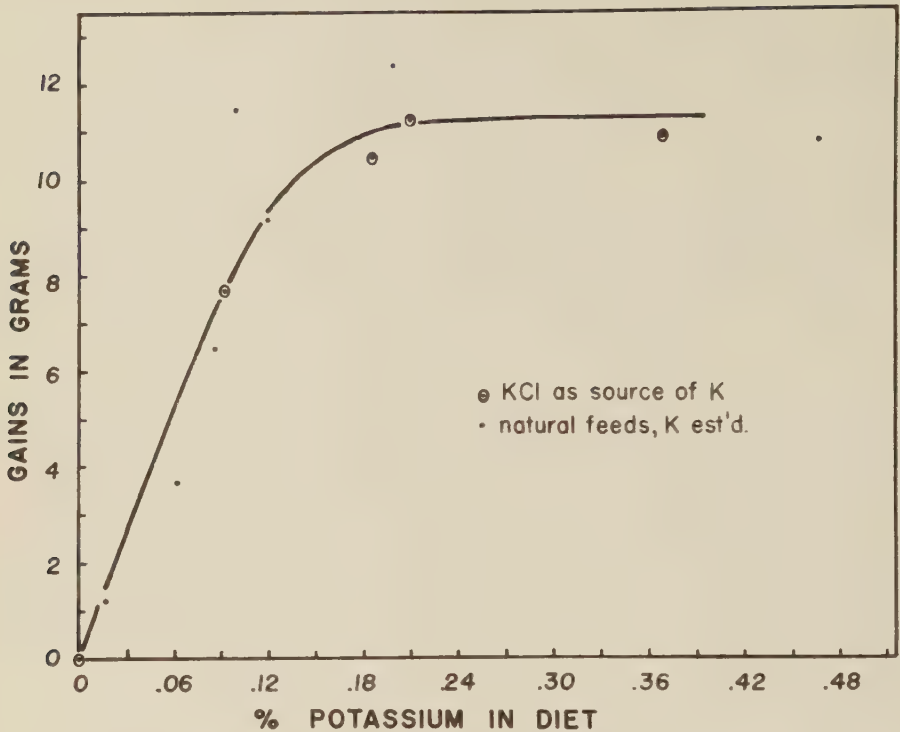


FIGURE 1. The relationship between growth rates in mice and level of potassium in the diet.

battery. Feed and water were allowed *ad libitum* and the laboratory temperature was maintained thermostatically at approximately 25°C. Gains and feed intakes of the mice were recorded for a 14-day period.

Potassium Supplements

KCl was added to obtain 0.99, 0.09 and 0.37 per cent potassium in the respective diets. Another diet was formulated by adding a supplementary mineral mixture to the basal ration to provide an additional 20 p.p.m. Co; 50 p.p.m. Mn; 5 p.p.m. F; 25 p.p.m. Zn, and 0.2 per cent K. Other test diets were made up by adding 5 per cent brewers' dried yeast; 5 per cent fish meal; 5 or 10 per cent wheat bran; 5 per cent dehydrated alfalfa meal; 10 per cent *Tricholoma nudum* residue* and *Tricholoma nudum* ash equivalent to 23.6 and 47.5 per cent of the dry residue.

RESULTS AND DISCUSSION

Mice fed the semi-purified diet with no supplementary source of potassium rapidly became emaciated and died, apparently from inanition, within 5 or 6 days after being put on test (Table 1). The inclusion of

* Courtesy of J. F. T. Spencer, National Research Council, Saskatoon, Sask., analysed 2 per cent potassium, dry basis.

TABLE 1.—POTASSIUM CONTENTS OF THE RATIONS FED AND THE 14-DAY WEIGHT GAINS MADE BY WEANLING MICE

Diet description	Potassium contents	Gains
	%	gm.
Purified diet, K-deficient	trace	0
+ 5% fish meal	.017*	1.2
+ 5% wheat bran	.062*	3.7
+ 5% brewers' dried yeast	.086*	6.5
+ 0.18 % KCl	.093	7.7
+ 5% dehydr. alfalfa meal	.100*	11.5
+ 10% wheat bran	.120*	9.2
+ 0.36% KCl	.186	10.5
+ 10% <i>Tricholoma nudum</i> residue	.200	12.4
+ Co, Mn, F, Zn, K supplement	.210	11.3
+ 0.72% KCl	.372	10.9
+ <i>Tricholoma nudum</i> ash	.471	10.8
+ <i>Tricholoma nudum</i> ash	.950	9.2

* Potassium contents estimated

any of the crude feedstuffs listed above permitted survival and when the potassium contents, according to Morrison's tables of average composition, were compared with the KCl supplements it became evident that the success of the natural feedstuffs might have been due to their contents of potassium. Since a maximum of 10 per cent of natural carrier of potassium was included in any diet, and since the basal diet was designed to provide sufficient safety margins in energy, protein, vitamins and all essential minerals except potassium, it is unlikely that the added feedstuff would stimulate growth other than through the medium of potassium.

The relationship between the potassium content of the diet and the growth rates is shown in Figure 1, which places emphasis upon the diets having known potassium contents and from which it is apparent that the potassium requirement of the normal growing mouse is approximately 0.2 per cent of the ration. This level thus agrees well with those found for the rat and the chick.

REFERENCES

1. Bell, J. M., J. D. Erfle, J. F. T. Spencer, and F. Reusser. *Tricholoma nudum* as a source of amino acids vitamins, and unidentified factors. Can. J. Animal Sci. 38: 122-128. 1958.
2. Davis, G. K., and J. K. Loosli. Mineral metabolism (animal). Annual Rev. Biochem. 23: 459-480. 1954.
3. Graw, C. R., F. H. Kratzer, and W. E. Newlon. Principles of nutrition for chickens and turkeys. Circ. 450, Calif. Agr. Expt. Sta. 1956.
4. Morrison, F. B. Feeds and feeding. 22nd ed. Morrison Pub. Co., Clinton, Iowa. 1956.

EFFECTS OF LOW FLUCTUATING TEMPERATURES ON FARM ANIMALS.

III. INFLUENCE OF AMBIENT AIR TEMPERATURE ON FEED INTAKE OF LACTATING HOLSTEIN-FRIESIAN COWS

M. A. MACDONALD¹ AND J. M. BELL²

University of Saskatchewan, Saskatoon, Saskatchewan

[Received for publication March 31, 1958]

ABSTRACT

This report presents effects of low temperatures on the feed consumption and efficiency of milk production of six mature, lactating, Holstein-Friesian cows that were confined in stanchions for three fortnightly experimental periods during which ambient temperatures measured in degree-hours per day (d-h/day) ranged from 110 to 1152 and daily minimum ambient air temperature (DMAAT) varied from 0° to 38°F. Applying results obtained, it was calculated that as temperatures decreased, i.e., d-h/day increased from 100 to 1200 and DMAAT decreased from 40° to 0°F, average daily intakes of total dry matter, hay, and gross and digestible Calories increased approximately 6.4 lb., 5.3 lb., 13 Therms and 9 Therms, respectively. Each of these increases was statistically significant at the 1 per cent level. Reductions in temperature also decreased gross and net caloric efficiencies of milk production approximately 10 and 8.5 per cent, respectively. These decreases were significant at the 2 per cent level. No correlation was evident between crude protein utilization and temperature.

Results indicated that thermal stress was not overcome adequately by supplementary hay intake alone and that appetite stimulation by low temperatures had a carry-over effect continuing at least 24 hours. For continued efficient milk production during winters where low ambient temperatures are prevalent these results suggest it is necessary to provide some form of building insulation, ambient heat and/or provide a high energy supplement to otherwise adequate production rations.

INTRODUCTION

The overriding influence of climate on feed and water supplies for animals needs no emphasis. In temperate latitudes the provision of accommodation and feed for maintaining production during cold weather often dominates livestock enterprises.

Low environmental temperatures stimulate appetites (13, 25) and increase metabolic rates. Increased caloric intakes improve cold tolerance by reducing the withdrawal rates on depot fat and by delaying the onset of fatigue from prolonged shivering. Activity increases heat production, thus relieving thermal stress in the cold but beyond this little can be said with confidence regarding the acclimatization of farm animals to cold (13).

While many experimental reports have been published on the feeding and winter management of livestock very few experiments were designed to measure the direct effects of low temperatures on milk production. Seale and Lapp (24) compared milk production and feed intake of dairy cattle kept indoors with those kept outdoors and while the latter group ate seven pounds more roughage per day there were no temperature details published.

¹ National Research Council of Canada Post-Doctorate Fellow; now Department of Animal Husbandry, Macdonald College, Que.

² Professor, Head, Department of Animal Husbandry, University of Saskatchewan, Saskatoon, Sask.

Missouri workers (20) indicated that hay consumption increased 40 to 80 per cent as temperatures declined from 50°F. to 8°F. They also reported that sudden decreases in temperature increased hay and T.D.N. intakes even more markedly (22). Changes in relative humidity at 40°F. and 12°F. did not affect T.D.N. or water consumption, or milk production of lactating dairy cows (21).

The availability of more precise information on the disposal of feed energy under low temperature conditions would facilitate the design of housing and of feeding systems for livestock. This paper presents effects of low fluctuating temperatures on feed consumption and feed efficiency in mature, lactating Holstein-Friesian cows.

MATERIALS AND METHODS

The data used in this report were obtained using Holstein-Friesian cows of the University of Saskatchewan dairy herd. All were maintained under the experimental conditions described previously (15, 16). Six cows in the declining phase of lactation were confined in portable stanchions on the manure pack for periods of approximately 14 days in January, February, and March, 1957. An interim period of approximately 14 days separated each test period. All animals were weighed at the commencement and termination of each test period. Hay was provided *ad libitum* to each cow and consumption was determined daily. Cold water was available to each animal according to conditions outlined previously (16). Concentrates were fed approximately according to N.R.C. requirements (19).

Caloric content of feedstuffs and feces from digestion trials was determined using a Parr oxygen-bomb calorimeter (4). Nitrogen content was determined by micro-Kjeldahl (9) and crude protein estimates were obtained by multiplying nitrogen content by 6.25. Digestibility of hay and hay plus concentrate rations was determined with Holstein-Friesian steers in metabolism stalls, at moderate ambient temperatures using the procedures recommended by Lloyd, Peckman, and Crampton (14). Water content of feedstuffs was determined by drying representative aliquots to constant weight (3). Levels of statistical significance were determined according to Fisher and Yates (8).

RESULTS AND DISCUSSION

Each of the following analyses represents 41 daily records on each of six experimental cows, or a total of 246 individual observations. The individual values recorded for each factor for each temperature category in Table 1 are averages of 6 to 24 observations. As in previous publications (15, 16), the term "degree-hours per day" has been abbreviated for convenience to d-h/day and the term "daily minimum ambient air temperature" has been abbreviated to DMAAT.

Effect of Temperature on Dry Matter Intake per Day

Dry matter intakes during the three periods of confinement for each cow were obtained by adding the weights of dry concentrate, dry beet pulp, and dry hay consumed daily (Table 1). As d-h/day increased and

TABLE 1.—AVERAGE NUTRIENT INTAKES AND EFFICIENCIES OF MILK ENERGY AND PROTEIN PRODUCTION AT VARIOUS LEVELS OF DEGREE HOURS PER DAY

d-h/day	Daily mean temperature	Intake per Day				Hay intake during subsequent day	Efficiency of Milk Production								
		Total dry matter	Total dry matter per 1000 lb. body weight	Total caloric	Digestible caloric		Hay	Gross caloric	Net caloric	Gross crude protein	Digestible crude protein	Net protein			
	°F	pounds	pounds	Therms	Therms	pounds	pounds	%	%	%	%	%	%	%	%
1152	2.0	48.8	34.9	96.1	59.8	27.7	27.6	14.5	18.6	22.0	29.6	36.5			
1142	2.4	48.6	34.7	96.9	60.3	28.3	25.3	14.3	18.4	22.7	30.1	37.2			
1132	2.8	50.0	35.7	97.8	60.8	28.8	26.8	15.4	19.6	22.5	30.4	37.5			
1102	4.1	47.7	34.1	95.9	59.6	27.6	24.5	14.4	18.4	25.4	34.2	42.2			
1052	6.2	45.0	32.1	87.9	54.8	27.2	29.8	14.5	19.1	25.6	34.3	42.9			
1042	6.6	47.1	33.7	92.7	57.7	25.8	23.0	15.1	19.5	26.9	36.2	44.9			
1002	8.2	42.9	30.6	84.0	52.4	21.0	22.8	16.8	22.4	25.7	34.3	43.3			
992	8.7	46.1	32.9	90.4	56.3	24.6	—	15.6	20.4	24.9	33.3	41.5			
982	9.1	43.6	31.2	85.4	53.3	21.8	21.0	15.9	21.1	25.6	34.2	43.0			
972	9.5	45.9	32.8	89.9	56.0	24.3	24.6	16.2	21.2	26.3	35.2	44.0			
882	13.2	49.8	35.6	97.6	60.6	26.1	27.1	14.5	18.5	22.2	30.0	36.9			
852	14.5	47.0	33.5	91.9	57.2	25.5	26.5	14.6	19.0	22.9	30.7	38.2			
832	15.3	47.3	33.8	92.5	57.6	26.0	26.8	14.7	19.0	22.0	29.5	36.7			
822	15.7	48.0	34.3	94.3	58.7	26.8	28.6	14.7	19.0	22.8	30.8	38.1			
782	17.4	44.6	31.8	87.8	54.7	23.0	—	15.4	20.2	27.2	36.4	45.6			
641	23.3	42.6	30.5	84.7	52.7	22.5	24.7	15.6	20.8	23.6	31.7	40.3			
631	23.7	44.5	31.8	87.2	54.4	22.8	23.6	17.7	23.3	26.5	35.4	44.4			
591	25.4	40.3	28.8	78.9	49.3	18.2	22.3	16.8	22.9	26.6	35.3	45.1			
551	27.0	42.9	30.6	79.4	49.6	18.6	21.4	16.9	23.0	24.5	32.5	41.5			
541	27.5	45.1	32.2	88.3	54.9	24.6	25.6	14.7	19.3	23.5	31.7	40.1			
531	27.9	44.5	31.8	86.9	54.0	24.4	23.8	14.6	19.2	24.8	33.6	42.5			
501	29.1	46.7	33.4	91.3	56.7	26.1	20.5	14.2	18.5	22.1	29.9	37.4			
471	30.4	45.3	32.3	88.6	55.2	23.6	26.2	18.2	24.0	25.8	34.6	43.3			
451	31.2	40.2	28.8	78.9	49.3	18.3	22.2	17.4	23.7	26.6	35.3	45.1			
411	32.9	41.2	29.4	80.7	50.4	19.3	18.8	16.8	22.8	24.8	33.0	42.0			
371	34.5	44.8	32.0	87.6	54.5	24.0	24.6	15.0	19.7	23.2	31.2	39.4			
290	37.9	43.0	30.7	84.1	52.3	22.8	23.7	15.3	20.4	22.3	30.0	38.2			
250	39.6	42.4	30.3	83.0	51.6	22.2	22.6	14.9	20.0	22.5	29.9	38.8			
230	40.4	43.5	31.1	85.0	52.8	23.3	—	13.9	18.5	22.1	30.9	38.0			
170	42.9	42.5	30.3	83.1	51.6	22.2	22.8	14.9	20.0	22.9	27.6	39.4			
110	45.4	43.8	31.3	85.7	53.3	23.3	23.3	14.0	18.6	20.4	—	35.0			
mean															
679.3	21.7	45.08	32.20	88.51	55.18	24.11	24.12	15.25	20.08	23.80	34.09	40.17			

Average nutrient intakes and efficiencies of milk energy and protein production at various levels of daily minimum ambient air temperature proximate the values reported in Table 1. They may be obtained upon request from the senior author.

TABLE 2.—REGRESSIONS OF VARIOUS NUTRIENTS (Y) ON TOTAL DEGREE HOURS PER DAY AND DAILY MINIMUM AMBIENT AIR TEMPERATURES (X)

Analysis	Factor regressed	Regressions on d-h/day				Regressions on DMAAT			
		df.	r	byx	Significance level	df.	r	byx	Significance level
1	Total dry matter intake per day	39	.587	.00594	1%	39	-.570	-.15051	1%
2	Total dry matter intake per day per 1000 lb. live weight	39	.642	.00421	1%	39	-.628	-.10750	1%
3	Gross caloric intake per day	39	.626	12.443	1%	39	-.604	-313.33	1%
4	Digestible caloric intake per day	39	.645	7.7779	1%	39	-.622	-195.69	1%
5	Hay intake per day	39	.501	.00509	1%	39	-.471	-.12500	1%
6	Hay intake during subsequent day	39	.411	.00409	1%	39	-.375	-.10025	2%
7	Gross efficiency of milk production	39	-.372	-.00141	2%	39	.363	.03593	2%
8	Net efficiency of milk production	39	-.290	-.00174	7%	39	.267	.04179	10%
9	Percentage crude protein recovered in milk	39	.006	—	N.S.	39	-.008	—	N.S.
10	Percentage digestible crude protein recovered in milk	39	-.025	—	N.S.	39	.026	—	N.S.
11	Net efficiency of protein recovered in milk	39	-.078	—	N.S.	39	.064	—	N.S.

DMAAT decreased; i.e. as temperatures became colder, there was a highly significant increase in total dry matter intake per day (Table 2). Employing the regression coefficients in Table 2 it was calculated that average dry matter intake per day increased from 41.6 lb. at 100 d-h/day to 48.2 lb. at 1200 d-h/day. Similarly average dry matter intake per day increased from 41.7 lb. on days with a DMAAT of 40°F. to 47.8 lb. on days with a DMAAT of 0°F. It was found that deviations from 40°F. to temperatures in the order of 1200 d-h/day or 0°F. DMAAT, were associated with dry matter intake increases of approximately 6 to 9 per cent. In spite of activity of the experimental animals being restricted by stanchioning, there was no evidence of shivering but appetite was stimulated by cold temperatures.

Effect of Temperature on Total Dry Matter Intake per Day per 1000 lb. Live Weight

Employing data from many sources, Winchester and Morris (27) calculated the effect of ambient temperature on dry matter consumption of several classes of livestock. They found that intakes of dry matter per 1000 lb. live weight of cows of European breeds plateaued at approximately 31.7 lb. daily between 70°F. and 40°F.

Regressions of dry matter consumed per 1000 lb. live weight on d-h/day and DMAAT were both highly significant (Table 2). Average dry matter consumed daily for the three intake periods was 32.2 lb. Upon applying regression coefficients it was found that at a mean daily temperature of 40°F. the average daily dry matter consumption was 30.4 lb. while at a temperature of 0°F. consumption was 34.4 lb. per day. It is interesting that the finding of Winchester and Morris (27) for lactating dairy cows was very close to the regressed value obtained here and that dry matter consumption per 1000 lb. live weight increased as temperature decreased in contrast to the plateau illustrated by these workers.

Effect of Temperature on Hay Consumption per Day

Missouri research workers (20) found that a reduction in mean temperature from 50°F. to 8°F. increased hay consumption by 40 to 80 per cent. A similar trend was evident in the present experiment as consumption rates increased 26.5 and 23.6 per cent, respectively, over the d-h/day and DMAAT ranges studied. The regressions of hay consumption by Holstein-Friesian cows on d-h/day and DMAAT at this institution were highly significant (Tables 1 and 2). Daily hay consumption increased from 21.2 lb. at 100 d-h/day (a mean temperature of 46°F.) to 26.8 lb. at 1200 d-h/day (a mean temperature of 0°F.). Similarly, daily hay consumption increased from 21.4 lb. at 40°F. DMAAT to 26.4 lb. at 0°F. DMAAT.

Effects of temperature on the metabolic activity of micro-organisms are well known (23). The effects of cold water ingestion on digestion rates of hay and on time of passage should be determined in order that limits may be established for hay as a source of heat for homeothermy in ruminants in cold environments.

Regressions of pounds of feed and calories consumed per day on d-h/day and DMAAT have been illustrated in Figures 1 and 2, respectively. It may be seen from the relatively large standard deviations and standard

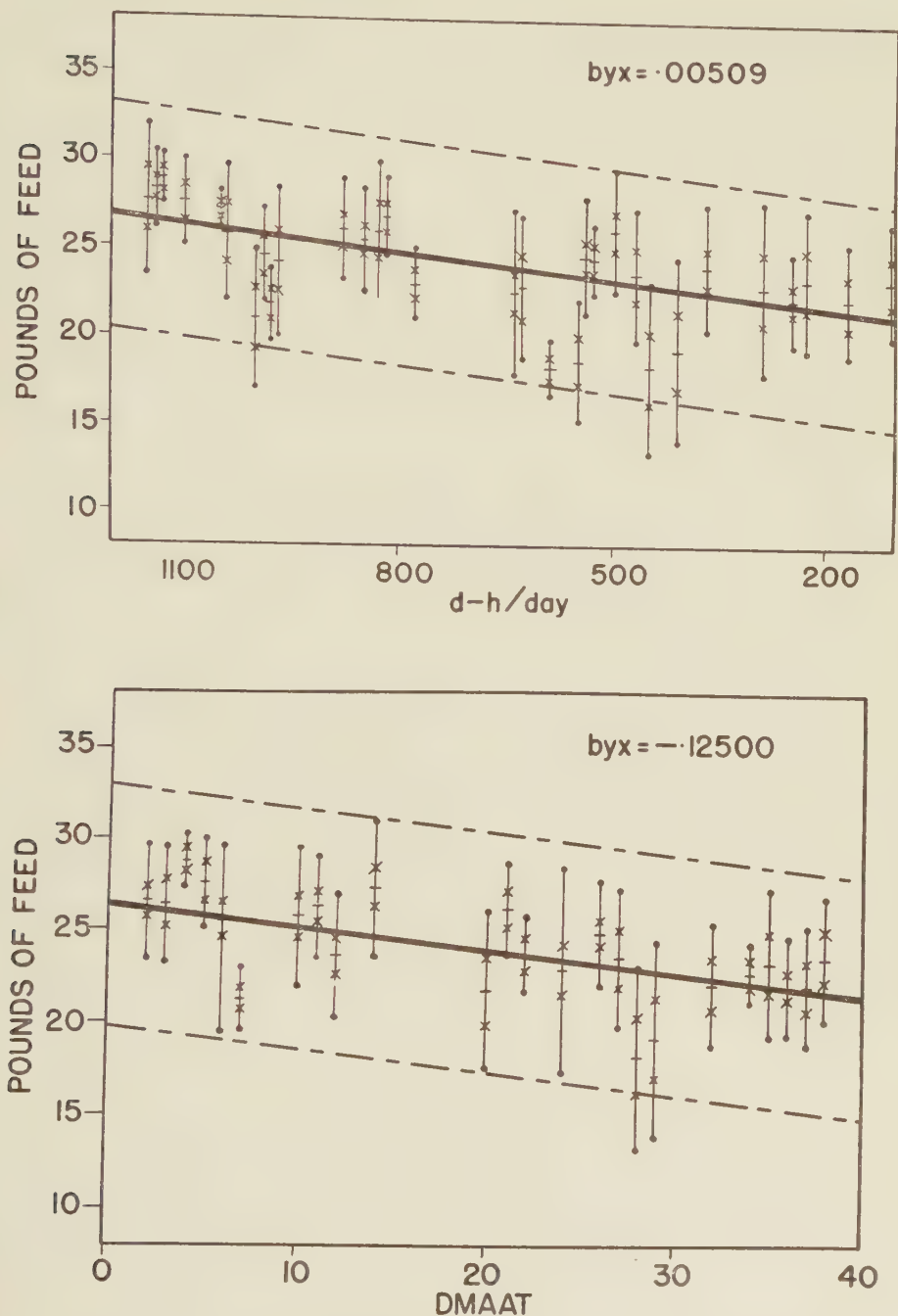


FIGURE 1. Relationship between temperature represented by d-h/day and DMAAT (X) and pounds of feed consumed daily per cow (Y). Legend: ● standard deviation, X standard error, — mean, — — — — standard deviation $\times 2$.

errors of the mean that individuals varied considerably in intake at most temperatures. This was to be expected since the experimental cows differed in body weight and level of milk production.

Effect of Temperature on Hay Consumption during the Subsequent Day

The fluctuating temperatures and *ad libitum* hay feeding regimen of this experiment permitted a study of temperature carryover effects on roughage intakes. The effects on water intakes have been reported (16) and water intakes, in turn, have been shown to be highly correlated with feed and energy intakes (1, 16). In this study it was found that as temperatures decreased hay consumption the *subsequent* day increased. The regressions of hay consumption on d-h/day and DMAAT were significant at 1 and 2 per cent levels, respectively. It is thus apparent that physiological adjustments to environmental temperature changes were not only detectable the first day but extended significantly into the following day and possibly longer.

It has been shown (5) that increased adrenal secretions result from short periods of exposure to cold. It is doubtful, however, that these hormones would cause any sustained effect upon appetite or cold tolerance because they elicit calorogenic responses of short duration. It seems more probable that the thyroid is directly responsible for increasing appetite, hence carry-over effects might be anticipated.

Effect of Temperature on Caloric Intake per Day

Feed samples were collected for analysis during each of the three confinement periods and representative lots of feed were used in a digestion trial. The results of the digestion trial appear in Table 3. Caloric intake values for beet pulp were estimated from Morrison's tables (18) and by using the factor 4.40 to convert grams T.D.N. to digestible Calories* (7). Digestion trials using 1000-lb. Holstein-Friesian steers were conducted at moderate temperatures only. Therefore, in calculating intakes of digestible Calories three assumptions must be made: First, that the digestibility coefficients for Holstein-Friesian steers and lactating cows are the same; second, that an animal's ability to digest feedstuffs is neither enhanced nor suppressed by low temperatures; third, that digestibility of feedstuffs remains constant within the intake range of this experiment.

* Crampton, E. W. *Private communication*. 1957.

TABLE 3.—DIGESTION TRIAL RESULTS

Feedstuff	% Moisture	Gross calories/gm. dry matter	Dry matter % digestible	Calories % digestible	Nitrogen % digestible
Hay.....	8.97	4.29	61.6	60.6	66.6
Beet pulp.....	9.04	4.16	—	—	—
Concentrate.....	9.21	4.42	66.8	66.9	86.7

Since hay consumption, the predominant variable in the ration, was significantly influenced by decreasing temperatures it is not surprising that average caloric intake was also influenced by decreasing temperatures. Upon regressing daily total caloric intake on d-h/day and DMAAT, it was found that as temperature decreased caloric intake increased. Both regressions were significant at the 1 per cent level and are graphically illustrated in Figure 2.

Average total energy intake increased from approximately 81.3 Therms per day at 100 d-h/day to 95.0 Therms per day at 1200 d-h/day. Similarly caloric intake increased from approximately 81.7 Therms to 94.2 Therms per day as DMAAT decreased from 40°F. to 0°F. The percentage changes in caloric intakes were approximately 17 and 15 per cent for the two temperature ranges studied.

Regressions of estimated daily digestible caloric intake on d-h/day and DMAAT were both significant at the 1 per cent level. In both cases estimated digestible caloric intake increased as temperature decreased (Table 2). Estimated intakes of digestible Calories increased from 50.4 to 59.1 Therms as d-h/day increased from 100 to 1200 while estimated intakes increased from 50.8 to 59.9 Therms as DMAAT decreased from 40°F. to 0°F.

Effect of Temperature on Caloric Efficiency of Milk Production

Gross caloric efficiencies were calculated by dividing the energy in milk produced by the gross caloric content of the feedstuffs ingested after adjusting for liveweight change by conventional methods (6, 26). Milk composition data are presented in a later paper of this series. All cows were in the declining phase of lactation and of average producing ability, hence efficiencies fell short of those estimated for good cows (6) but the efficiencies were comparable to those reported for Jersey and Holstein-Friesian cows employed in low temperature studies in Missouri (22).

After adjusting for the decrease in efficiency with time, which was highly significant ($df = 39$, $r = .444$), it was found that as temperature decreased the gross caloric efficiency of milk production also decreased. Regressions of both gross efficiency of milk production on d-h/day and DMAAT were statistically significant at the 2 per cent level (Table 2). As d-h/day increased from 100 to 1200 gross efficiency decreased from 15.98 to 14.43 per cent, and as DMAAT decreased from 40°F. to 0°F. gross efficiency decreased from 16.03 to 14.59 per cent. Decreases in efficiency with decreasing temperature within the d-h/day and DMAAT ranges studied were 10.74 and 9.87 per cent, respectively. These results are in general keeping with the conclusion by others (11), that gradually decreasing temperatures from 50°F. to 9°F. caused an increase in heat production of 20 to 30 per cent in lactating Holstein cows. The increased heat production was associated with decreased milk production and some increase in feed consumption (22). The authors concluded (11) that extra heat produced (hence the decrease in efficiency) was the result of increased muscular tone and/or increased specific dynamic action of feed. In other studies the Missouri workers found that decreasing the temperature from 50°F. to 9°F. increased heat production more in tropical than in temperate breeds of cattle (12) and that there was no appreciable effect on that heat production of large, high-producing Holsteins (28).

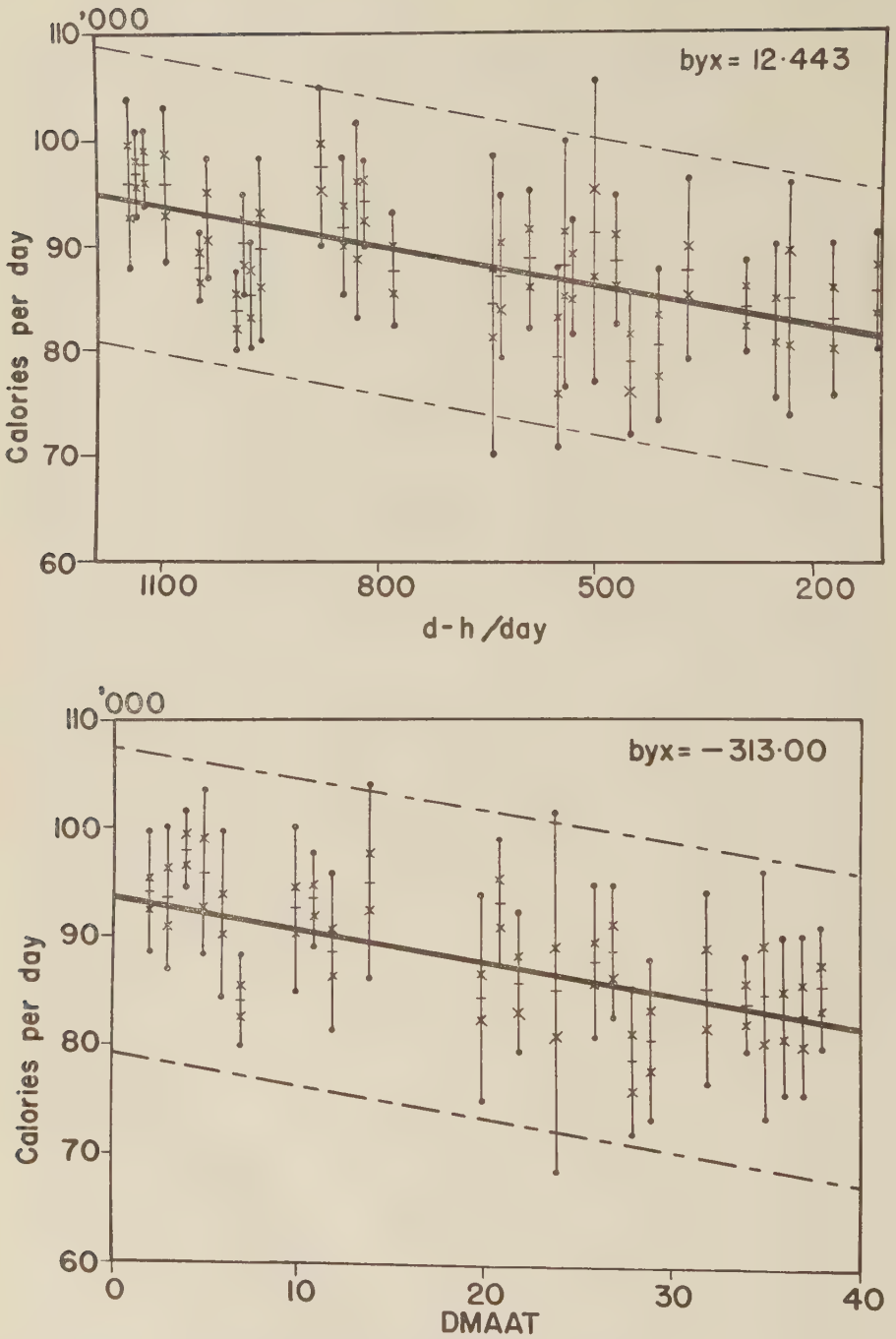


FIGURE 2. Relationship between temperature represented by d-h/day and DMAAT (X) and Calories consumed daily per cow (Y). Legend: ● standard deviation, × standard error, — mean, — — — — standard deviation $\times 2$.

Estimates of net efficiency of milk production are hazardous, since they necessitate the deduction of maintenance energy requirements (2, 6). Unfortunately, the term maintenance infers an exactness which biologically it does not have in practice. The energy requirement for maintenance is the minimum amount needed to keep an animal in energy equilibrium (17) and no adjustment is made in standards for thermal stress since environmentally neutral conditions are assumed in such calculations. Furthermore, since stimulation of appetite with declining temperature influences intakes during the subsequent day, true daily net efficiencies are rather difficult to approach and, since these factors influence net efficiencies, both within and between days and specific temperatures, estimates of statistical significance are less clearly definable. This is clearly illustrated in the net caloric efficiency estimates of regression which appear in Table 2. These were obtained by applying mathematical adjustments for maintenance estimates based on body weight and changes in body weight during the experimental periods. Regressions of net caloric efficiency of milk production estimates on d-h/day and DMAAT were statistically significant only at the 7 and 10 per cent levels, respectively. Applying the regression values, cognizant of the low level of statistical significance, it was found that net caloric efficiency decreased as temperature decreased. As d-h/day increased from 100 to 1200 net caloric efficiency of milk production decreased from 20.99 to 19.07 per cent while as DMAAT decreased from 40°F. to 0°F. it decreased from 20.97 to 19.32 per cent.

Effect of Temperature on Efficiency of Protein Utilization for Milk Production

It is of interest to know the effects of environmental temperature upon efficiency of utilization of protein as well as energy. The protein allowances in current feeding standards for dairy cattle are based on findings obtained within the zone of thermal neutrality; hence it is possible that they might differ under conditions of low temperature.

Gross protein efficiencies on a daily basis were computed by dividing the crude protein content of milk produced by the crude protein content of feedstuffs consumed. Values were corrected for stage of lactation. Following this, regressions of protein intake on d-h/day and DMAAT were calculated and it was found (Table 2) that the percentage recovery in milk of crude protein ingested was independent of temperature changes.

Similar results were obtained with *digestible* protein recovery. It is recalled, however, that protein digestibility values were subject to the same limitations discussed above with regard to energy.

Calculated *net* protein efficiencies (maintenance allowance deducted) likewise were unaffected by temperatures in the ranges studied.

It is evident from this experiment that declining temperatures ranging from 38°F. to 0°F. in DMAAT and 110 to 1152 in d-h/day were associated with increased consumption of feed as expressed by dry matter, hay, gross energy, or digestible energy. Associated with these changes in intake was a decreased efficiency of conversion of feed energy into milk energy. No effect was demonstrated, however, with regard to efficiency of protein utilization. Thus, it is obvious that the nutritional requirements of these cows were influenced by environmental temperatures and it is equally

apparent that if the nutrient requirements of farm livestock managed under conditions of low temperature are to be clearly and precisely defined, maintenance and digestibility studies must be undertaken in low temperature, not thermo-neutral environments.

REFERENCES

1. Adolph, E. F. The metabolism and distribution of water in body and tissues. *Physiol. Rev.* 13:336-371. 1933.
2. Armsby, H. P. The nutrition of farm animals. Macmillan Co., New York, N.Y. 1928.
3. Association of Official Agricultural Chemists. Official methods of analysis. 7th ed. Washington, D.C. 1950.
4. Bell, J. M. A comparison of peroxide- and oxygen-bomb calorimetry of feedstuffs. *Can. J. Agr. Sci.* 35:366-370. 1955.
5. Best, C. H., and N. B. Taylor. The physiological basis of medical practice. 5th ed. Williams and Watkins, Baltimore, Md. 1950.
6. Brody, S. Bioenergetics and growth. Reinhold Pub. Corp., New York, N.Y. 1945.
7. Crampton, E. W., L. E. Lloyd, and V. G. MacKay. The caloric value of TDN. *J. Animal Sci.* 16:541-545. 1957.
8. Fisher, R. A., and F. Yates. Statistical tables for biological, agricultural, and medical research. 2nd ed. Oliver and Boyd, Edinburgh, Scotland. 1943.
9. Hawk, P. B., B. L. Oser, and W. H. Summerson. Practical physiological chemistry. 12th ed. Blakiston Co., Toronto, Ont. 1951.
10. Kelly, M. A. R., and I. W. Rupel. Relation of stable environment to milk production. U.S.D.A. Tech. Bull. 591. 1937.
11. Kibler, H. H., and S. Brody. Environmental physiology with special reference to domestic animals. VII. Influence of temperature, 50° to 5°F. and 50° to 95°F. on heat production and cardiorespiratory activities of dairy cattle. Missouri Expt. Sta. Research Bull. 450. 1949.
12. Kibler, H. H., and S. Brody. Environmental physiology with special reference to domestic animals. XI. Effects of temperature 50° to 105°F. and 50° to 9°F. on heat production and cardiorespiratory activities in Brahman, Jersey, and Holstein cows. Missouri Expt. Sta. Research Bull. 464. 1950.
13. Lee, D. H. K. Heat and cold. *Ann. Rev. Physiol.* 10:365-386. 1948.
14. Lloyd, L. E., H. E. Peckham, and E. W. Crampton. The effect of change of ration on the required length of preliminary feeding period in digestion trials with sheep. *J. Animal Sci.* 15:846-853. 1956.
15. MacDonald, M. A., and J. M. Bell. Effects of low fluctuating temperatures on farm animals. I. Influence of ambient air temperature on the respiration rate, heart rate, and rectal temperature of lactating Holstein-Friesian cows. *Can. J. Animal Sci.* 38:10-22. 1958.
16. MacDonald, M. A., and J. M. Bell. Effects of low fluctuating temperatures on farm animals. II. Influence of ambient air temperature on water intake of lactating Holstein-Friesian cows. *Can. J. Animal Sci.* 38:23-32. 1958.
17. Maynard, L. A. Animal nutrition. 3rd ed. McGraw-Hill Book Co. Ltd., Toronto, Ont.
18. Morrison, H. B. Feeds and feeding. 20th ed. Morrison Pub. Co., Ithaca, N.Y. 1947.
19. Nutrient requirements for domestic animals. III. Nutrient requirements of dairy cattle. National Research Council, Washington 4, D.C.
20. Ragsdale, A. C., H. J. Thompson, D. M. Worstell, and S. Brody. Environmental physiology with special reference to domestic animals. IX. Milk production and feed and water consumption responses of Brahman, Jersey, and Holstein cows to changes in temperature, 50° to 105°F. and 50° to 8°F. Missouri Expt. Sta. Research Bull. 460. 1950.
21. Ragsdale, A. C., H. J. Thompson, D. M. Worstell, and S. Brody. Environmental physiology and shelter engineering with special reference to domestic animals. XXI. The effect of humidity on milk production and composition, feed and water consumption, and body weight in cattle. Missouri Expt. Sta. Research Bull. 521. 1953.

22. Ragsdale, A. C., D. M. Worstell, H. J. Thompson, and S. Brady. Environmental physiology with special reference to domestic animals. VI. Influence of temperature, 50° to 0°F. and 50° to 95°F. on milk production, feed and water consumption and body weight in Jersey and Holstein cows. Missouri Expt. Sta. Research Bull. 449. 1949.
23. Salle, A. J. Fundamental principles of bacteriology. 7th ed. McGraw-Hill Book Co., New York, N.Y. 1943.
24. Seale, M. E., and H. Lapp. Loose housing of dairy cattle. Proc. University of Manitoba. 7th Ann. Livestock Day, pp. 1-6. 1957.
25. Wilkinson, Doug. Land of the long day. Clarke, Irwin, Ltd., Toronto, Ont. 1955.
26. Williams, S. B., and P. E. Sylvestre. A method of measuring the relative production of pastures with livestock. Proc. 7th Pacific Sci. Congress 6:246-250. 1949.
27. Winchester, C. F., and M. J. Morris. Water intake rates of cattle. J. Animal Sci. 15:722-740. 1956.
28. Worstell, D. M., and S. Brody. Environmental physiology and shelter engineering with special reference to domestic animals. XX. Comparative physiological reactions of European and Indian cattle to changing temperature. Missouri Expt. Sta. Research Bull. 515. 1953.

EFFECTS OF LOW FLUCTUATING TEMPERATURES ON FARM ANIMALS

IV. INFLUENCE OF TEMPERATURE ON MILK YIELD AND MILK COMPOSITION

M. A. MACDONALD¹ AND J. M. BELL²

University of Saskatchewan, Saskatoon, Saskatchewan

[Received for publication July 7, 1958]

ABSTRACT

Changes in milk yield and milk composition from mature Holstein-Friesian cows managed under low fluctuating environmental temperatures have been presented. The experiment conducted at the University of Saskatchewan, Saskatoon, lasted for 106 days during the winter of 1956-57. Loafing barn temperatures, measured continuously in degree-hours per day (d-h/day) ranged from 110 to 1202 and daily minimum ambient air temperature (DMAAT) varied from -5° to 38° F.

As temperature decreased below 25° F., daily yield of milk decreased significantly. Milk yield was unaffected by d-h/day at mean temperatures over 25° F. (600 d-h/day) and on days colder than 600 d-h/day the temperature effect on milk yield was curvilinear. The rate of decline in milk yield (pounds) was four times greater on days when DMAAT was below 10° F. than on days when DMAAT was above 10° F. While DMAAT had a significant effect on milk total-solids percentage and a highly significant negative effect on crude protein yield, the effect of d-h/day on these two factors was not significant. Neither DMAAT nor d-h/day had a significant effect on butterfat yield and percentage, fat-corrected milk yield, total-solids yield, solids-not-fat yield and percentage, or crude protein percentage.

Stage of lactation had a significant effect on all characteristics studied except butterfat percentage.

Under conditions of declining lactation and a high, variable plane of nutrition, cows that had been subjected to gradually cooling variable low temperatures were influenced by low daily minimum temperatures (DMAAT) to a greater degree than low mean daily temperatures (d-h/day).

INTRODUCTION

Reviews of the effect of low environmental temperature on milk production and composition by Hancock (15) and Cobble and Herman (4) indicate the lack of published experiments in this field. Psychrometric room data indicate that low temperatures in the ranges from 50° to 4° F. and 50° to 8° F. resulted in milk yield decreases in Jersey cows of 4 to 6 lb. per day when temperatures were reduced gradually. When Jersey cows were subjected to sudden temperature decreases, the reduction in milk yield increased (24, 26). In Holstein cows milk yield was only slightly affected by a temperature decrease (24, 26). Under similar circumstances there was a 10 to 35 per cent increase in butterfat percentage in Jerseys but in Holsteins the butterfat percentage increase was lower (4). Cobble and Herman (4) also found that total solids were significantly greater at temperatures below freezing than at 50° F., solids-not-fat tended to increase parallel with butterfat percentage and total nitrogen increased substantially as temperature decreased below 40° F.

¹ National Research Council of Canada Post-Doctorate Fellow; now Department of Animal Husbandry, Macdonald College, Que.

² Professor, Head, Department of Animal Husbandry, University of Saskatchewan, Saskatoon, Sask.

In field trials in which low temperatures fluctuated, the results are more difficult to appraise; the majority of research results indicate that cows kept at low temperatures undergo decreases in milk yield. Davis (5) found that a drop in ambient temperature in an open or a closed stable caused a decrease in milk yield in spite of increased feed consumption at lower temperatures. Kelley and Rupel (18) subjected lactating cows to an exposure period of 5 days at an average temperature of 18° F. They also found a decrease in milk yield accompanying the reduction in temperature. Finally, Sementovskaya and Garkavi [(cited by Hancock (15))] recorded a reduction of 26 per cent in milk yield and bodyweight losses of 2 lb. per day when cows were exposed to low temperatures without supplemental feeding. However, with a 25 per cent increase in feed allowance, both milk yield and bodyweight were maintained.

Other workers also reported experiments in which no reduction in milk yield accompanied the decrease in ambient temperature. Dice (6, 7, 8) concluded that shelter from wind, rain, and snow was sufficient to maintain milk production in cows not permitted additional feed. However, under these conditions heifers grew more slowly than conventionally-housed heifers. Kelley and Rupel (18) also concluded, using moderate temperature data, that cold weather generally affects cows little if they are well fed and are acclimatized to open pens. Witzel and Barrett (32) found that daily changes in atmospheric temperature did not cause fluctuations in milk production of cows in an open shed. In a later study Witzel and Heizer (33) found fat-corrected-milk (FCM) production of cows in a cold barn to be slightly less than that produced by cows housed in a warm barn.

As indicated in reviews of literature by Cobble and Herman (4) and Hancock (15), research results have consistently indicated that low ambient temperatures cause an increase in butterfat percentage. In the studies quoted by these reviewers, Brooks (3) kept cows alternately in moderate (50° to 55° F.) and "low" (30° to 40° F.) barn temperatures. Ragsdale and Turner (25) and Hays (17) gathered data in the temperature range of 27° to 72° F. Even Kelley and Rupel's (18) short-duration experiment at an average ambient temperature of 18° F. was conducted under experimental temperatures which exceed outside average winter temperatures in much of agricultural Canada (29). It is apparent that caution must be used in accepting and attempting to apply in Canada research results from experiments where the comparative term "low temperature" is not qualified by the exact temperatures under which the results were obtained and the conclusions drawn.

In this paper some direct and indirect effects of fluctuating, low ambient temperatures on yield and composition of milk from Holstein-Friesian cows are presented.

MATERIALS AND METHODS

Data used for this study were from cows of the University of Saskatchewan dairy herd. They were maintained under experimental conditions described previously (19,20,21). All six experimental cows were in the declining phase of lactation. Each was milked twice daily in a milking

TABLE 1.—AVERAGE DAILY MILK YIELD AND COMPOSITION OF MILK AT VARIOUS LEVELS OF DEGREE HOURS PER DAY (D-H/DAY) AFTER ADJUSTING FOR STAGE OF LACTATION

d-h/day Range	Mean d-h/day	Av. daily temper- ature °F	Daily yield in pounds				Percentage of daily milk yield in pounds					
			Milk	Fat corrected milk	Butterfat	Total solids	Solids- not- fat	Crude protein	Butterfat	Total solids	Solids- not- fat	Crude protein
1200-1249	1202	0.0	45.8	—	—	—	—	—	—	—	—	—
1150-1199	1152	2.0	49.8	43.9	1.51	5.50	4.07	1.38	3.29	12.33	8.77	3.33
1100-1149	1124	3.2	43.7	41.1	1.50	5.00	3.57	1.36	3.56	12.27	8.61	3.27
1050-1099	1052	6.2	41.3	—	—	—	—	—	—	—	—	—
1000-1049	1016	7.7	46.6	42.5	1.48	5.31	3.86	1.42	3.40	12.31	8.77	3.23
950-999	984	9.0	46.7	42.5	1.51	5.20	3.71	1.37	3.49	12.15	8.56	3.16
900-949	910	12.1	46.4	43.3	1.54	5.30	3.80	1.38	3.56	12.49	8.68	3.25
850-899	871	13.7	46.5	41.3	1.43	5.10	3.72	1.34	3.29	11.91	8.55	3.34
800-849	832	15.3	47.6	43.0	1.49	5.27	3.83	1.40	3.35	12.04	8.48	3.09
750-799	770	17.9	46.8	43.0	1.52	5.24	3.69	1.50	3.52	12.50	8.65	3.42
700-749	724	19.9	47.6	44.2	1.59	5.35	3.86	1.48	3.70	12.80	8.76	3.38
650-699	671	22.0	47.1	46.4	1.71	5.59	3.98	1.48	3.91	13.16	8.79	3.29
600-649	622	24.1	45.9	42.1	1.51	5.13	3.68	1.34	3.59	12.47	8.65	3.15
550-599	570	26.2	47.0	42.7	1.52	5.17	3.66	1.41	3.55	12.47	8.41	3.26
500-549	522	28.2	47.1	42.1	1.48	5.14	3.74	1.38	3.47	12.39	8.61	3.19
450-499	463	30.7	46.3	42.6	1.53	5.13	3.68	1.36	3.62	12.41	8.59	3.15
400-449	420	32.5	47.3	42.5	1.52	5.17	3.72	1.40	3.61	12.54	8.70	3.38
350-399	371	34.5	48.1	41.3	1.41	5.67	3.69	1.39	3.39	12.25	8.53	3.22
300-349	338	35.9	47.5	—	—	—	—	—	—	—	—	—
250-299	272	38.7	47.8	41.9	1.46	5.05	3.72	1.33	3.41	12.32	8.49	3.05
200-249	230	40.4	45.9	40.1	1.39	4.80	3.54	1.34	3.39	12.27	8.43	3.18
150-199	170	42.9	46.4	40.7	1.43	4.85	3.55	1.33	3.42	12.22	8.35	3.10
100-149	110	45.4	45.7	40.6	1.43	4.85	3.54	1.25	3.52	12.47	8.50	2.97
Mean	—	—	46.7	42.3	1.49	5.18	3.72	1.38	3.49	12.34	8.59	3.22

Average daily milk yield and composition of milk at various levels of DMAAT somewhat approximate the values appearing in Table 1 and may be obtained on request from the senior author.

parlour and daily yields during the 106-day experimental period, extending from mid-December, 1956 to April 1, 1957, were analysed. All cows were on R.O.P.; daily milk production records were kept according to Canada Department of Agriculture regulations. Representative milk samples were collected at each milking during periods of stanchion confinement and at both milkings every second day during interim periods. After collection, milk samples were cooled to 40° F. Determinations of specific gravity and butterfat percentage were made within 18 hours after milking. Aliquots for nitrogen determinations were placed in watertight plastic bags, sealed and frozen for subsequent analysis. To obtain daily yields, results of separate analysis of morning and evening milk samples were used. Determinations of specific gravity, total solids, and solids-not-fat were made according to procedures recommended by the U.S. Milk Industry Foundation (22). FCM values were determined according to Rice, Andrews, and Warwick (27) and caloric content of milk samples was determined using Brody's formula (2) and checked by calorimetric analysis in this laboratory. Nitrogen content was determined using a micro-Kjeldahl and distillation technique (16) and crude protein content was calculated employing the conventional factor ($N \times 6.25$). The standard Babcock procedure was used to determine butterfat percentages (22). For statistical analyses a regression of milk component yield on time was calculated and an adjusted yield obtained using the regression equation. Adjusted values were then used for further regressions on temperature. Levels of statistical significance were determined using values presented by Fisher and Yates (13).

RESULTS AND DISCUSSION

The purpose of this and the studies reported previously (19,20,21) was to measure some effects of low fluctuating temperatures on mature lactating Holstein-Friesian cows. Definition of the direct effect of temperature is complicated by variations in plane of nutrition and in metabolism in the experimental animals during the period of study. For example, it is well known that after peak milk production has been reached there is an extended period of lactation during which there is a steady decline in daily milk yield (23). There are also concomitant changes in milk composition, feed intake, and endocrine and metabolic activity of the cows. It is possible, for two main reasons, that the full effect of temperature on milk production was minimized. First, all experimental cows were permitted to compensate for low temperatures by increasing feed intake (21). Second, the regression used to adjust for stage of lactation assumes that all the temperature-induced suppression of milk production was subsequently recovered. It is generally believed by dairymen that it is easy to suppress milk production and almost impossible to completely re-establish production rates following suppression. Thus, these results represent the influence of temperature on recoverable milk yield and composition for which increased hay consumption could not compensate.

Effect of d-h/day and DMAAT on Adjusted Daily Milk Yield

Time-adjusted average daily milk yield in various ranges of d-h/day appears in Table 1. Averages represent 6 to 60 individual observations

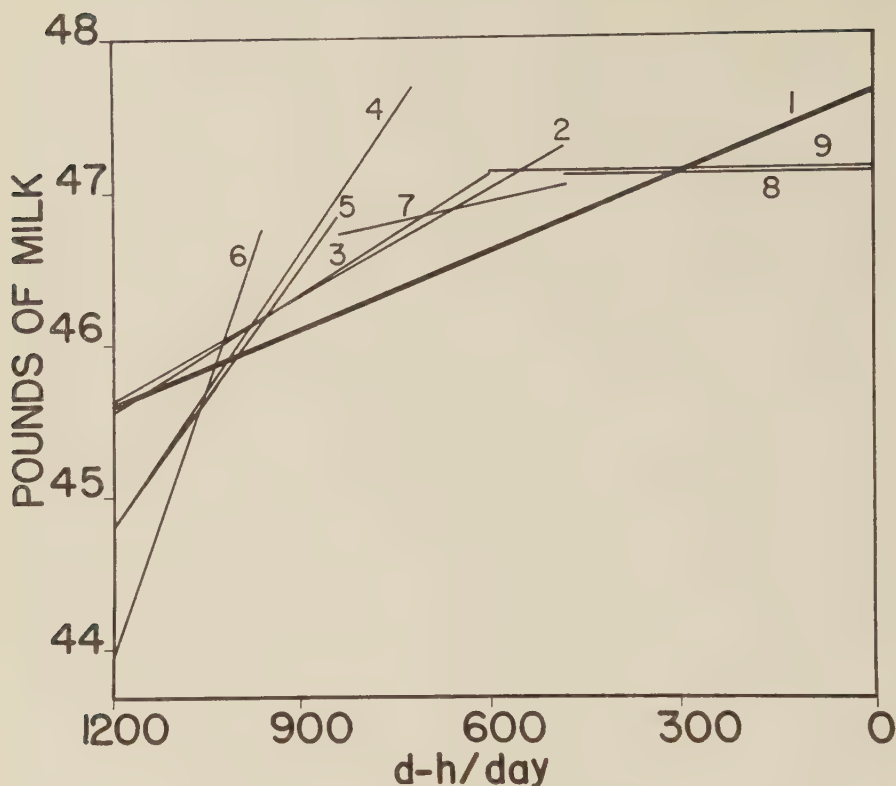


FIGURE 1. Regressions of degree-hours per day (d-h/day) on average daily milk yield. Numbers on the regression lines correspond to analysis numbers in Table 2.

in each category. Upon regressing adjusted daily milk yield on d-h/day, the result was significant at the 2 per cent level for the 1200 to 0 d-h/day range. It was calculated that at 0 d-h/day (50 °F. mean temperature) milk yield was 47.62 lb. per day, and at 1200 d-h/day (0° F. mean temperature) it was 45.58 lb. per day—a reduction of 2.04 lb. of milk.

On plotting the various regressions of adjusted milk yield on d-h/day (Figure 1), it is evident that the over-all regression (Table 2, Analysis 1) exaggerates expected milk yield at both 1200 and 0 d-h/day. The true effect of temperature represented by d-h/day on milk production is curvilinear, not linear. By increasing energy intake at low temperatures through increased hay consumption and possibly by using energy which would form a portion of the specific dynamic action (SDA) component at high environmental temperatures, cows were able to maintain milk production until average d-h/day reached approximately the 600 d-h level (25° F.). At colder temperatures milk yield was reduced in favour of the cows' homeothermy. The reduction in milk yield in the lower temperature ranges in spite of increased energy intakes (Tables 2, Analyses 4, 5, and 6) contradicts somewhat the results obtained by Sementovskaya and Gorkavi [(quoted by Hancock (15))] in which, by increasing the feed allow-

TABLE 2.—REGRESSIONS OF ADJUSTED MILK YIELD PER DAY (V) ON D-H/DAY IN VARIOUS RANGES

Analysis	Regression of adjusted milk yield on d-h/day range	Calculated range of milk yield /day	r	d.f.	b _{yx}	Level of significance
1.	1200 to 0 d-h/day (0° to 50° F)	45.58-47.62	-.233	103	-.00170	2%
2	1200 to 480 d-h/day (0° to 30° F)	45.62-47.28	-.226	73	-.00231	6%
3	1200 to 600 d-h/day (0° to 25° F)	45.55-47.05	-.195	56	-.00251	N.S.
4	1200 to 720 d-h/day (0° to 20° F)	44.78-47.67	-.318	44	-.00582	4%
5	1200 to 840 d-h/day (0° to 15° F)	44.77-46.83	-.223	30	-.00572	N.S.
6	1200 to 960 d-h/day (0° to 10° F)	43.89-46.75	-.449	17	-.11910	6%
7	840 to 480 d-h/day (15° to 30° F)	46.72-47.23	-.094	41	-.00139	N.S.
8	480 to 0 d-h/day (30° to 50° F)	47.09-47.10	-.012	27	-.00020	N.S.
9	600 to 0 d-h/day (25° to 50° F)	47.13-47.13	+.002	44	.00002	N.S.

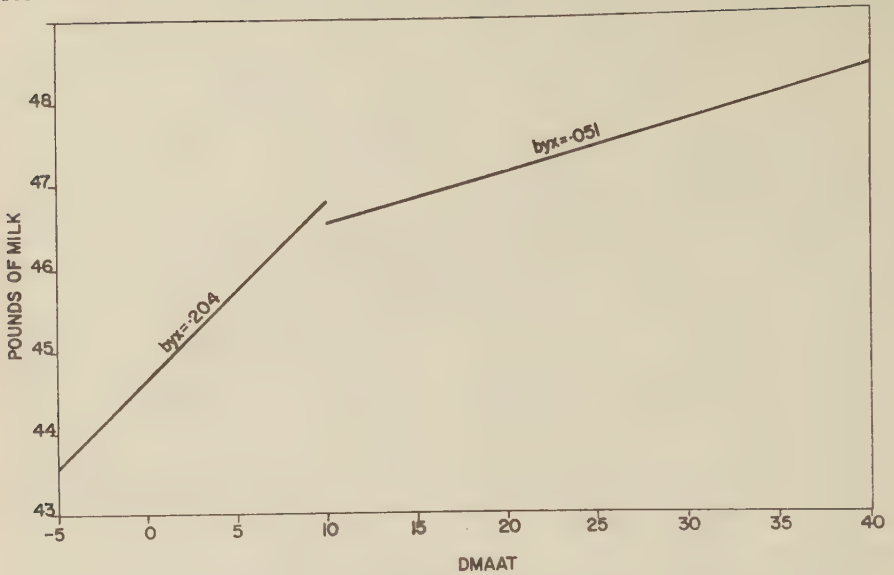


FIGURE 2. Regressions of daily minimum ambient air temperature (DMAAT) on average daily milk yield in temperature ranges of -5° to 10° F. and 10° to 40° F.

ance, they were able to maintain both milk yield and body weight at temperatures which otherwise resulted in milk yields declining by 26 per cent and in body weight losses.

Cobble and Herman (4) found that Holsteins showed no decrease in milk yield at low temperatures except during a period of 2 weeks when the temperature was suddenly decreased to 4° F.

As DMAAT decreased adjusted daily milk yield also decreased (Figure 2). The regression of adjusted milk yield on DMAAT ($byx = 0.059$) in the range of -5° to 40° F. was significant at the 1 per cent level.

In contrast to analyses of nutritional and physiological interrelationships with temperature reported previously (19, 20, 21), average milk yield was more highly correlated with DMAAT than with d-h/day. This indicates that milk production is influenced by the extreme low in temperature to a greater extent than the daily mean temperature while for factors such as heart and respiration rates, rectal temperature, feed and water intake, and milk production efficiencies, the reverse is true.

Effect of d-h/day and DMAAT on Butterfat Yield and Butterfat Percentage

Research results reviewed earlier generally agree that low temperatures increase butterfat percentage. Most do not indicate whether the increase is sufficiently large to offset temperature-induced reductions in milk yield in order to maintain butterfat yield. Other investigations have shown that plane of nutrition influences both butterfat yield and percentage (10, 30, 31) and that stage of lactation also influences the composition of milk (9, 11, 12, 23). For example, Cobble and Herman (4) reported increases of 10 to 35 per cent in butterfat percentage at low temperatures. At this institution, the free choice of hay intake minimized the effects of temperature on butterfat production and percentage. These values and all values

for milk components presented in subsequent sections represent the influence of temperature on recoverable yield and percentage for which changes in feed intake could not compensate.

The decrease in butterfat yielded per day with time was significant at the 1 per cent level. After adjusting for the effect of time, the regression of butterfat yield on neither d-h/day nor DMAAT was significant. It must be concluded that the increased feed consumption at temperatures in the range studied in this experiment was sufficient to maintain butterfat production even though insufficient to maintain milk yield.

Throughout the 106-day experimental period, the average butterfat percentage increase with time was not significant. In contrast with many studies reviewed earlier, the relationship between adjusted butterfat percentage and both d-h/day and DMAAT was non-significant (Table 3). It is obvious that to establish the exact effects of temperature *per se* on butterfat yield and percentage at low temperatures, it is essential that stage of lactation, plane of nutrition, and other principal factors affecting these milk characteristics be carefully controlled.

Effect of d-h/day and DMAAT on Fat-corrected-milk (FCM) Yield

Fat-corrected-milk (FCM) yield which combines milk yield and butterfat yield into a single factor decreased with time during the experimental period. This is expected since both milk yield and butterfat yield decreased with time. After adjusting for stage of lactation, the regressions of FCM on both d-h/day and DMAAT were statistically non-significant (Table 3).

Effect of d-h/day and DMAAT on Total-solids Yield and Total-solids Percentage

The total-solids portion of milk comprises butterfat plus solids-not-fat. During latter stages of lactation total-solids yield decreases with time (23). After adjusting for the decline in total-solids yield of the experimental cows, it was found that temperature was not significantly correlated with total-solids yields (Table 3, Analyses 8 and 9). In contrast, adjusted percentage total-solids increased significantly as DMAAT increased. Since both adjusted milk yield and adjusted percentage total solids increased significantly with increases in DMAAT, it is rather unexpected to find that total-solids yield was not significantly affected by temperature. Cobble and Herman (4) also reported an increase in total solids yield as temperatures declined. Further work must be undertaken using more accurate techniques to resolve this anomaly in results.

Effect of d-h/day and DMAAT on Solids-not-fat (SNF) Yield and SNF Percentage

Stage of lactation appears to affect the yield of solids-not-fat in much the same manner as it affects milk yield (23). Plane of nutrition is also known to affect the SNF content of milk (14, 28). As in other studies, there were significant differences in solids-not-fat yield with time and between cows in this study. It is not surprising that after adjusting for stage of lactation, neither solids-not-fat nor percentage solids-not-fat were significantly influenced by temperature. Rowland (28) reported the

TABLE 3.—REGRESSIONS OF BUTTERFAT PERCENTAGE, FCM, TOTAL-MILK-SOLIDS, TOTAL-SOLIDS PERCENTAGE, SNF, SNF PERCENTAGE, DAILY CRUDE PROTEIN YIELD, AND CRUDE PROTEIN PERCENTAGE (Y) ON EXPERIMENTAL DAYS, (X) D-H/DAY (\bar{x}_1) AND DMAAT (\bar{x}_2)

Analysis	Regression of:	r	d.f.	byx	Level of significance
1	Butterfat % on experimental day	.062	58		N.S.
2	Adjusted butterfat % on d-h/day	-.054	58	—	N.S.
3	Adjusted butterfat % on DMAAT	.112	58	—	N.S.
4	Fat-corrected-milk (FCM) yield on experimental day	-.713	58	-.07745	1%
5	Adjusted FCM yield on d-h/day	.143	58	—	N.S.
6	Adjusted FCM yield on DMAAT	-.094	58	—	N.S.
7	Total solids yield on experimental day	-.414	58	-.00676	1%
8	Adjusted daily total solids yield on d-h/day	.160	58	—	N.S.
9	Adjusted daily total solids yield on DMAAT	-.072	58	—	N.S.
10	% total solids on experimental day	.244	58	.00329	7%
11	Adjusted % total solids on d-h/day	-.161	58	—	N.S.
12	Adjusted % total solids on DMAAT	.263	58	.08549	4%
13	Solids-not-fat (SNF) on experimental day	-.595	58	-.00587	1%
14	Adjusted SNF yield per day on d-h/day	.020	58	—	N.S.
15	Adjusted SNF yield per day on DMAAT	-.016	58	—	N.S.
16	% SNF on experimental day	.376	58	.00349	1%
17	% SNF on d-h/day	.181	58	—	N.S.
18	% SNF on DMAAT	-.147	48	—	N.S.
19	Crude protein (CP) yield on experimental day	-.637	58	-.00298	1%
20	Adjusted CP yield on d-h/day	.141	58	—	N.S.
21	Adjusted CP yield on DMAAT	-.534	58	-.00436	1%
22	% CP on experimental day	-.090	58	—	N.S.
23	Adjusted % CP on d-h/day	.207	58	—	N.S.
24	Adjusted % CP on DMAAT	-.164	58	—	N.S.

solids-not-fat content of milk to be lowest during the winter and attributed the depression to a low plane of nutrition. Nutrition was of less importance in the current study because hay was provided *ad libitum* permitting compensatory intakes of feedstuffs with which to offset effects of temperatures. Cobble and Herman (4) found that the solids-not-fat content of milk increased with decreasing temperature.

Effect of d-h/day and DMAAT on Crude Protein Yield and Crude Protein Percentage

During the course of this study crude protein yield per day decreased significantly (Table 3), but stage of lactation did not significantly influence

crude protein percentage. After adjusting for time, only the effect of DMAAT on crude protein yield was statistically significant ($p < .01$). As DMAAT decreased, crude protein yield increased. Cobble and Herman (4) also found increases in total nitrogen content in both Jersey and Holstein milk at low environmental temperature. It was reported previously that animals ingested more crude protein on cold days than on more moderate days and that the efficiency of crude protein utilization for milk production was independent of temperature (21). It seems that, while cold temperatures necessitate utilizing additional ingested energy to maintain homeothermy, the additional crude protein fraction ingested concomitant with feedstuffs energy was not only surplus to possible additional requirements for crude protein balance but also was utilized for extra milk crude protein yield. Other studies have indicated that protein content of milk increased with starvation (14), underfeeding (10), and with stage of lactation (1).

This series of studies (19,20,21) has revealed that under housing conditions where the minimum ambient air temperatures ranged from -5° to 38° F., there were significant effects of low temperatures upon heart rates, rectal temperatures, respiration rates, feed intakes, water intakes, total volume of milk produced, and efficiency of energy conversion with regard to milk production. It is also apparent that various responses of the cows were influenced to a greater extent by the low extremes of daily temperatures than by the average daily temperatures. This may indicate that, at least within certain yet undefined temperature limits, rapid variations of temperature are more difficult to offset by the homeothermic mechanism than are steady temperatures outside the zone of thermal neutrality. These studies have provided physiological evidence that dairy cows were under thermal stress at temperatures near 0° F. and that 10° F. appeared to be critical with respect to milk yield (uncorrected for fat percentage). Below 10° F. the rate of decline in milk volume in relation to lowering temperatures was significantly faster than above this temperature. Expressed in economic terms, the low temperatures encountered under loose housing in this experiment effected a reduction in gross revenue of approximately 3.5 per cent from cows producing about 11,000 lb. of milk testing 3.6 per cent butterfat.

While further work is required to elucidate the more precise relationships between energy utilization and ambient air temperatures, and while artificially heated or even well-insulated barns are unnecessary for lactating cows, it may be concluded that sufficient control should be exercised to minimize temperature fluctuations and to prevent temperatures from declining below 10° F. if maximum efficiency of milk production is desired.

REFERENCES

1. Azarme, E. Variations in the protein content of milk during lactation. *J. Dairy Research* 9:121-146. 1938.
2. Brody, S. *Bioenergetics and growth*. Reinhold Pub. Corp., New York, N.Y. 1945.
3. Brooks, W. P. Experiment in warming a stable for cows. *Mass. Hatch Sta. Rept.* 39. 1895.
4. Cobble, J. W., and H. A. Herman. The influence of environmental temperatures on the composition of milk of the dairy cow. *Missouri Expt. Sta. Research Bull.* 485. 1951.
5. Davis, H. P. The effect of open-shed housing as compared with the closed stable for milk cows. 19th Annual Rept. Penna. Sta. Coll. Agr. Expt. Sta. 1913-14.

6. Dice, J. R. The nutrients required by dairy cows kept in an open shed vs. cows kept in a dairy barn. *J. Dairy Sci.* 18:447-448. 1935.
7. Dice, J. R. The influence of stable temperature on the production and feed requirements of dairy cattle. *J. Dairy Sci.* 23:61-69. 1940.
8. Dice, J. R. The ability of yearling heifers to withstand cold temperatures. *J. Dairy Sci.* 25:678-679. 1942.
9. Drakeley, T. J., and M. K. White. The joint influence of the period of lactation and the age of the cow on the yield and quality of the milk. *J. Agr. Sci.* 18:496-506. 1928.
10. Eckles, C. H., and L. S. Palmer. Influence of plane of nutrition of the cow upon the composition and properties of milk. *U.S. Bur. Animal Ind. Bull.* 157. 1916.
11. Eckles, C. H., and R. H. Shaw. The influence of the stage of lactation on the composition and properties of milk. *U. S. Bur. Animal Ind. Bull.* 155. 1913.
12. Eckles, C. H., and R. H. Shaw. Variations in the composition and properties of the individual cows. *U. S. Bur. Animal Ind. Bull.* 157. 1913.
13. Fisher, R. A., and F. Yates. Statistical tables for biological, agricultural, and medical research. 2nd ed. Oliver & Boyd, Edinburgh. 1943.
14. Gowen, J. W., and E. R. Tobey. Studies of milk secretion. The influence of inanition. *J. Gen. Physiol.* 15:45-66. 1931.
15. Hancock, J. The direct influence of climate on milk production. *Dairy Sci. Abstr.* 16: Review article 26. 1954.
16. Hawk, P. B., B. L. Oser, and W. H. Summerson. Practical physiological chemistry. 12th ed. Blakiston Co., Toronto, Ont. 1951.
17. Hays, W. P. The effect of environmental temperature on the percentage of fat in cows' milk. *J. Dairy Sci.* 9:219-235. 1926.
18. Kelley, M. A. R., and I. W. Rupel. Relation of stable environment to milk production. *U.S.D.A. Tech. Bull.* 591. 1937.
19. MacDonald, M. A., and J. M. Bell. Effects of low fluctuating temperatures on farm animals. I. Influence of ambient air temperature on respiration rate, heart rate, and rectal temperature in lactating Holstein-Friesian cows. *Can. J. Animal Sci.* 38:10-22. 1958.
20. MacDonald, M. A., and J. M. Bell. Effects of low fluctuating temperatures on farm animals. II. Influence of ambient air temperature on water intake of lactating Holstein-Friesian cows. *Can. J. Animal Sci.* 38:23-32. 1958.
21. MacDonald, M. A., and J. M. Bell. Effects of low fluctuating temperatures on farm animals. III. Influence of temperature on feed intake of lactating Holstein-Friesian cows. *Can. J. Animal Sci.* 38:148-159. 1958.
22. Milk Industry Foundation. Laboratory manual: Methods of analysis of milk and its products. Milk Industry Foundation, Washington, D.C. 1949.
23. Nicholson, W. S., Jr., W. R. Thomas, H. S. Willard, and D. C. Brown. Effect of stage of lactation on the solid-milk components of individual Holstein-Friesian cows. *Proc. Amer. Soc. Animal Prod. (Western Section)* 8:XII, 1-13. 1957.
24. Ragsdale, A. C., H. J. Thompson, D. M. Worstell, and S. Brody. Environmental physiology with special reference to domestic animals. IX. Milk production and feed and water consumption responses of Brahman, Jersey, and Holstein cows to changes in temperature 50° to 105° F. and 50° to 8° F. *Missouri Expt. Sta. Res. Bull.* 460. 1950.
25. Ragsdale, A. C., and C. W. Turner. The stage of lactation as a factor in the variation of the per cent in fat in cow's milk. *J. Dairy Sci.* 5:22-26. 1922.
26. Ragsdale, A. C., D. M. Worstell, H. J. Thompson, and S. Brody. Environmental physiology with special reference to domestic animals. VI. Influence of temperature, 50° to 0° F., and 50° to 95° F., on milk production, feed and water consumption, and body weight in Jersey and Holstein cows. *Missouri Expt. Sta. Research Bull.* 449. 1949.
27. Rice, V. A., F. N. Andrews, and E. J. Warwick. Breeding better livestock. McGraw-Hill Book Co., Toronto, Ont. 1953.
28. Rowlands, S. J. The occurrence in winter of milk with a low content of solids-not-fat. *J. Dairy Research* 13:261-266. 1944.
29. Thomas, M. K. Climatological atlas of Canada. Publ. 3151, National Research Council, Ottawa, Ont. 1953.
30. Turner, C. W. Factors affecting the composition of milk. *Missouri Agr. Expt. Sta. Bull.* 365. 1936.
31. White, G. C., and H. F. Judkins. Variations in fat, solids-not-fat, and total solids in cows' milk. *Storrs Agr. Expt. Sta. Bull.* 94. 1918.
32. Witzel, S. A., and G. R. Barrett. Dairy cow housing under study. *Agr. Eng.* 25: 83-89, 95. 1944.
33. Witzel, S. A., and E. E. Heizer. A five-year summary of dairy barn research. *Agr. Eng.* 27:479-505, 508. 1946.

DRY MATTER LOSS AND VOLATILE FATTY ACID PRODUCTION IN THE ARTIFICIAL RUMEN AS INDICES OF FORAGE QUALITY^{1,2}

J. M. ASPLUND³, R. T. BERG⁴, L. W. McELROY⁴ AND W. J. PIGDEN⁵

University of Alberta, Edmonton, Alberta

[Received for publication April 21, 1958]

ABSTRACT

Procedures are described for determining dry matter losses and for measuring volatile fatty acid production by direct chromatographic separation of acids produced during incubation of finely ground forages in miniature artificial rumen units. The results of preliminary assays done on three samples of unknown digestibility—excellent alfalfa hay, good mixed grass-alfalfa hay, and oat straw—showed that significantly different percentages of dry matter were lost and significantly different amounts of fatty acids were produced when feeds of such diverse quality were fermented. Analysis of the data for two groups of 11 and 6 samples of hay, for which dry matter digestibility coefficients as determined with sheep were available, indicated the existence of a high degree of correlation between dry matter digestibility *in vivo* and both dry matter loss and fatty acid production *in vitro*. Differences in assay results were observed when filtered rumen fluid or washed cells from the rumen fluid of sheep fed hay or from sheep fed straw were employed as sources of inocula. Examples of unsolved problems which are pertinent to the appraisal of the efficacy of artificial rumen assays for the estimation of the nutritive value of forages are discussed.

INTRODUCTION

Several types of artificial rumen have been devised to permit the study of rumen fermentation *in vitro*; Marston (10), Louw *et al.* (9), Burroughs *et al.* (3), Huhtanen *et al.* (7), and Pigden (12). These devices have been shown to be relatively reliable and have yielded valuable information concerning the digestion of cellulose and forages, and the utilization of urea in the rumen.

Limited evidence is available to suggest that the artificial rumen may be used as a means of assessing the relative quality or nutritive value of forages. Baumgardt and Hill (1) observed a loss of dry matter during *in vitro* fermentation which appeared to be related to the quality of the forages fermented. Pigden and Bell (13) demonstrated a significant correlation between anthrone carbohydrate digestion *in vitro* and the digestion of organic matter of forages *in vivo*. Moreover, according to Gray *et al.* (6) there is some evidence that the amount and nature of volatile fatty acid production *in vitro* may be related to forage quality. The results of studies on relationships which may exist between dry matter loss or volatile fatty acid production *in vitro* and dry matter digestibility *in vivo* are presented in the present paper.

¹ Joint contribution from the Department of Animal Science, University of Alberta, Edmonton, Alberta, and the Division of Animal and Poultry Science, Experimental Farms Service, Ottawa, Ontario.

² Supported in part by a grant from the National Research Council of Canada.

³ Graduate student, University of Alberta, 1955-57; present address, Department of Animal Husbandry, University of Wisconsin, Madison, Wis., U.S.A.

⁴ Department of Animal Science, University of Alberta, Edmonton, Alta.

⁵ Division of Animal and Poultry Science, Central Experimental Farm, Ottawa, Ont.

METHODS

Artificial Rumen Units

Miniature artificial rumen units containing 500 mg. of substrate, ground in a Wiley mill through a 40-mesh sieve, and 10 ml. of inoculum were prepared according to the method of Huhtanen *et al.* (7), except that the buffer solution was modified to include 2 to 3 crystals of CoCl_2 per 10 litres. The units were placed on 90-mm. watch glasses and incubated at 37.5°C .

Inocula

Mature sheep, each with a permanent plastic rumen cannula inserted by procedures described by Dougherty (5) and Phillipson and Innes (11), were used as sources of inocula. Inocula were prepared from rumen fluid taken from sheep 3 to 4 hours after they had been fed, by drawing the fluid into a suction flask attached to a water pump. *Filtered* inocula were prepared by filtering the freshly-drawn rumen fluid through two layers of cheesecloth, and were used within an hour of the time of collection. A considerable variation in their dry matter content was noted. *Washed cell* inocula were prepared essentially by the method of Cheng *et al.* (4). Filtered rumen fluid was centrifuged in 100-ml. tubes at 1000 r.p.m. for 3 to 5 minutes. The supernatant was decanted and centrifuged at 3000 r.p.m. for 35 minutes; the resulting sediment was suspended in a phosphate buffer at pH 7 and again centrifuged at 3000 r.p.m. for 35 minutes. The final sediment was re-suspended in a volume of artificial saliva buffer equal to one-half of the original volume of rumen fluid. Ten-ml. portions of this suspension were used immediately for inoculation.

Volatile Fatty Acids (V.F.A.)

After incubation, the liquid in the jar, plus rinsings from the outer surface of the sac, were transferred quantitatively to a 600-ml. beaker. Some fatty acid escaped from the fermentation chamber in the artificial saliva buffer which oozed out around the protruding end of the sac and dried on the outside of the jar. This dried material was collected by placing a watch glass under the fermentation chamber and rinsing the jar and watch glass with distilled water, following an overnight refrigeration period. The rinsings were added to the main body of the buffer bath in the 600-ml. beaker and the whole was made to 250 ml. with distilled water in a volumetric flask.

The V.F.A. present in a 50-ml. aliquot of this solution were separated and estimated by partition chromatography by a modification of the method of Keeney (8). The aliquot, in a 100-ml. beaker, was made alkaline by the addition of 0.5 ml. of 1 N KOH, covered with a watch glass and evaporated to dryness on a steam bath. The residue was dissolved in 2 ml. of 12 per cent by volume H_2SO_4 , and a mixture of this solution with 3 gm. silicic acid was transferred to a chromatographic column as described by Keeney.

In the preliminary trials values obtained for V.F.A. present in inoculum control units containing 10 ml. of filtered rumen fluid inocula and incubated at the same time as the test units were subtracted from test unit values to give 'net' yields as shown in Table 1. For reasons outlined under

Preliminary Trials, control units containing 10 ml. of inoculum, treated with 3 to 4 drops of 40 per cent formaldehyde, and refrigerated during the period of incubation of the test units, were used in subsequent experiments with feeds of known digestibility.

Dry Matter (D.M.) Loss

Weighed samples of finely ground forages were placed in weighed fermentation sacs after the forages and sacs had been dried for 12 hours at 65° C. and 20-lb. vacuum. The sacs were then inoculated and incubated in the artificial rumen. After incubation the sacs were removed, rinsed with distilled water and dialysed in tap-water at 12° to 14° C. for 24 hours. Following dialysis they were dried overnight at room temperature in an air stream, and then in an oven for 12 hours at 65° C. and 20-lb. vacuum before being weighed. The inoculum control sacs were prepared by placing 10 ml. of *filtered or washed cell* inoculum in a dried weighed sac, adding 2 or 3 drops of formaldehyde, and refrigerating in a jar of saliva buffer for the period of incubation of the test sacs. These sacs were washed, dialysed, dried and weighed with the test sacs.

PRELIMINARY TRIALS

Excellent alfalfa hay (18.9 per cent crude protein and 20.4 per cent crude fibre); good mixed grass-alfalfa hay (12.5 per cent crude protein and 26.2 per cent crude fibre), and oat straw (5.9 per cent crude protein and 32.1 per cent crude fibre) were used as substrates to determine whether, for feeds of distinctly diverse quality, differences could be measured in the weight of total V.F.A., in the weight of individual fatty acids produced, or in the loss of D.M. during fermentation in the artificial rumen. Effect of source of inocula was also studied. Two filtrates were prepared simultaneously from pooled rumen fluid from two sheep fed the mixed grass-alfalfa hay and from two fed the straw. These were designated as *hay* or *straw* inoculum and a mixture of equal parts of each as *combined* inoculum. Duplicate samples of the three substrates were incubated with each type of inoculum. Dry matter losses and V.F.A. yields were determined.

RESULTS

The data for 'net' total V.F.A. production, ratio of per cent acetic acid to per cent propionic acid, and loss of D.M., are presented in Table 1. Analysis of variance demonstrated highly significant differences between substrates and between inocula from different sources in 'net' total V.F.A. production and in D.M. loss. Differences between inocula with respect to proportions of acetic and propionic acids produced were highly significant ($P < 0.01$). The corresponding differences between substrates were not significant, but the fact that the mean square for interaction was highly significant indicates that substrates may have an effect on the proportions of acetic and propionic acids produced. The standard deviation for total V.F.A. production associated with differences between fermentations conducted with separate filtrates of a given type of rumen fluid was 2.9 mg., while that for D.M. loss was 2.1 per cent. The *combined* inoculum produced results intermediate between those of the other two inocula.

TABLE 1.—'Net' TOTAL VOLATILE FATTY ACID PRODUCTION, RATIO ACETIC TO PROPIONIC ACID¹ AND DRY MATTER LOSS AS AFFECTED BY SUBSTRATES AND INOCULA (24-HOUR INCUBATION)

substrate	Inocula from, filtrate	(1) Sheep fed hay			(2) Sheep fed straw			(3) Mixture of (1) and (2)		
		Total fatty acid ²	Ratio % acetic % propionic	Dry matter loss %	Total fatty acid ²	Ratio % acetic % propionic	Dry matter loss %	Total fatty acid ²	Ratio % acetic % propionic	Dry matter loss %
Alfalfa	1	215	3.0	63	166	1.4	55	190	1.7	56
	2	223	2.5	68	158	1.3	52	188	1.9	58
Mixed hay	1	188	2.9	58	100	1.2	48	147	1.9	54
	2	177	3.1	63	94	1.0	48	143	1.9	53
Straw	1	139	3.3	48	80	0.8	41	111	1.6	46
	2	144	3.2	46	85	0.8	42	127	1.6	43

¹ Acetic plus propionic acid accounted for 81.88 per cent of the total weight of fatty acids.

² Mg. per gm. substrate.

V.F.A. Inoculum Control

In the above preliminary work, 'net' V.F.A. yields in test units were calculated by subtracting from the gross V.F.A. present in such units the V.F.A. present in simultaneously incubated control units containing only filtered inoculum. If this procedure is to be accepted as valid, it is necessary to assume that both the rate and nature of fermentation of partially digested substrate present in filtered inocula are essentially the same in the control and test units. This assumption is unsound in theory. Results obtained in these trials, as summarized in Table 2, suggest that feed residues in filtered inocula probably undergo a different and more rapid fermentation in the absence than in the presence of fresh substrate.

TABLE 2.—ACIDS (ACETIC = A, PROPIONIC = P) IN TEST AND CONTROL UNITS, NET CALCULATED YIELDS, AND NET CALCULATED YIELDS AS DECIMAL FRACTIONS OF WEIGHTS OF ACIDS IN CONTROL UNITS

Substrate	Inoculum	Test unit		Control unit		Net		Net ÷ Control	
		A	P	A	P	A	P	A	P
		mg.	mg.	mg.	mg.	mg.	mg.		
Alfalfa	Hay	118	47	52	23	66	24	1.3	1.0
	Straw	83	39	44	11	39	28	0.9	2.5
Straw	Hay	98	38	52	23	46	15	0.9	0.6
	Straw	61	31	44	11	17	20	0.4	1.8

Thus it appeared that, in subsequent assays conducted on forages of known digestibility, correction only for quantities of V.F.A. present in 10 ml. of filtered inoculum at the time of inoculation might involve less error. These quantities were found to be negligible, in fact so small that it was difficult or impossible to determine them accurately. No further study was given to the problem of V.F.A. control, so that the data for V.F.A. in forages of known digestibility included in the following section are simply gross values for acids found in test units.

EXPERIMENTS WITH FORAGES OF KNOWN DIGESTIBILITY

Results obtained from artificial rumen assays conducted on two groups of 11 and 6 samples of forages, for which D.M. digestibility coefficients are available, are included in this section. The first group consisted of 11 hays which were fed to sheep and D.M. digestibility coefficients obtained by the total collection procedure and reported by Bell *et al.* (2). They were harvested in 1950 and stored in the ground state under refrigeration after August, 1951. The 6 samples in Group II were forages harvested in 1956 at Ottawa, frozen as harvested, and held in low-temperature storage until fed to sheep. Four of the D.M. digestibility coefficients as reported in Table 4 were obtained by the total collection procedure and the other two were estimated, using fecal nitrogen as an

TABLE 3.—GROUP I HAYS—MEAN ARTIFICIAL RUMEN ASSAY RESULTS AND SUMMARY OF STATISTICAL ANALYSES OF DATA FOR ELEVEN FORAGES HARVESTED IN 1950

Inocula From sheep fed	Dry Matter Loss, %												Crude protein % of dry matter ²	
	Gross total fatty acid production (mg./gm. substrate)		Filtered						Washed cell					Dry matter digestibility coefficient <i>in vitro</i> ¹ Y
	Filtered		Hay			Straw			Hay		Straw			
	Hay	Straw	X ₁ 18	X ₂ 24	X ₃ 48	X ₄ 18	X ₅ 24	X ₆ 48	X ₇ 24	X ₈ 48	X ₉ 24	X ₁₀ 48		
Incubation time, hr.	24	24												
<i>Kochia scoparia</i> , 1st stage	396	294	53	65	71	48	56	68	47	54	48	56	21.5	
Alfalfa	434	288	47	60	69	45	50	61	44	58	43	55	19.8	
Brome grass	362	276	36	50	59	33	41	55	32	42	33	49	13.4	
Intermediate wheatgrass	368	264	35	46	62	32	34	54	27	31	31	37	9.7	
Russian wild rye	334	242	29	43	58	26	32	53	25	36	29	43	13.4	
Oat hay	354	254	33	43	60	33	33	48	26	36	31	40	10.7	
Canadian wild rye	372	270	36	49	60	28	38	52	24	41	26	39	12.0	
Brome grass straw	354	258	33	42	59	27	34	53	25	38	26	38	9.4	
<i>Kochia scoparia</i> , 2nd stage	370	292	48	54	63	41	45	52	31	47	40	49	11.2	
<i>Kochia chadsu</i>	362	254	44	49	62	32	37	51	26	43	31	41	10.8	
Marsh hay	334	228	29	40	59	21	24	41	23	25	21	29	6.8	
Mean	367	265	38	49	62	33	39	53	30	41	33	43		
Correlation with dry matter digestibility coefficient	.71*	.66*	.48	.75**	.69*	.68*	.76**	.87**	.82**	.67*	.70*	.75**	.91**	
Regression coefficient	.13	.16	.30	.49	.83	.42	.43	.65	.52	.36	.45	.47	1.05	
Standard error of estimate	3.8	4.1	4.7	3.5	3.9	3.9	3.5	2.7	3.0	4.0	3.8	3.6	2.3	

¹ Bell *et al.* (2)² Pigden (12)

* Significant at P < 0.05

** Significant at P < 0.01

index of digestibility. On completion of the digestion trials surplus quantities were dried, ground, and stored under refrigeration until used in the present study.

Assays as summarized below were done on these hays:

Group I—1) total V.F.A. production during a 24-hour incubation period in artificial rumen units in triplicate after inoculation with *filtered hay* or *straw* inoculum; 2) D.M. loss during 18-, 24- and 48-hour incubation periods in artificial rumen units in duplicate after inoculation with *filtered* inocula as in 1); 3) D.M. loss during 24- and 48-hour incubation periods in artificial rumen units in duplicate after inoculation with *washed cell* inocula. Inocula were prepared from rumen fluid collected from pairs of sheep fed hay or straw as described above.

Group II—D.M. loss during 24- and 48-hour incubation periods in artificial rumen units in duplicate after inoculation with *washed cell* inoculum prepared from pooled rumen fluid collected from 3 cannulated sheep fed a ration of good mixed grass-alfalfa hay.

RESULTS

The assay data for samples in Group I, and part of the results of statistical analyses done on these data are summarized in Table 3.

Fatty Acids

With respect to total V.F.A. production it may be noted that, as in the preliminary trials, total acid production was appreciably higher for inocula from sheep fed hay than for those from sheep fed straw. Coefficients of correlation with D.M. digestibility *in vivo* were significant ($P < 0.05$) for the results of assays done with both types of inocula. Differences between substrates with respect to ratios of acetic/propionic acid yields approached significance at $P < 0.05$ and the mean square for substrates \times inocula interaction was significant at $P < 0.01$. For the 11 hays the mean ratios acetic/propionic acid produced were 2.7 and 2.5 for the *hay* and *straw* inocula respectively. These differences between *hay* and *straw* inocula with regard to relative yields of the two acids were quite consistent and significant at $P < 0.01$.

The fact that corresponding inocula differences recorded in the preliminary trials (Table 1) were much larger than those observed for any of the 11 forages in Group I is, as has been indicated under 'Preliminary Trials', attributable mainly to marked differences in the nature of the results obtained when 'net' rather than gross yields of V.F.A. are used. The desirability of employing *washed cell* inocula to study possible effects of sources of inocula on proportions of different fatty acids produced in the artificial rumen is indicated.

Dry Matter Loss

To study the possibility that incubation time might influence the degree of correlation between results for D.M. loss in the artificial rumen and the D.M. digestibility coefficients of these forages as determined with sheep, assays were conducted to determine D.M. loss at incubation times of

12, 18, 24 and 48 hours with *filtered hay* and *straw* inocula, and at 24 and 48 hours with *hay* and *straw washed cell* inocula. Results obtained at 12 hours were highly variable and are not reported. As is shown in Table 3, correlation coefficients between values for D.M. loss in the artificial rumen and *in vivo* digestibility coefficients of the forages were, with the exception of that for the 18-hour period with *hay* inoculum, either significant or highly significant.

From a comparison of the data in Table 3 for *filtered* vs. *washed cell* inocula it is interesting to note that: 1) employment of *washed cell* inocula did not materially increase the degree of association between *in vitro* and *in vivo* data for D.M. digestibility; 2) when filtered inocula were used, D.M. losses were consistently greater when inocula from sheep fed hay were used than when those from sheep fed straw were employed, whereas with *washed cell* inocula essentially the reverse situation existed.

The results of an analysis of covariance on the data in Table 3 for D.M. digestibility *in vitro* and *in vivo* showed no significant difference among the 10 regression coefficients, indicating that correlation and regression coefficients, based on Y values and pooled data for D.M. loss, columns X_1 - X_{10} , would be most reliable for prediction purposes. These pooled coefficients, 0.71 and 0.45, indicate that about 50 per cent (r^2) of the variation in the X's is associated with variation in Y, and on the average an increase of 1 per cent in X corresponds to an increase of approximately 0.5 per cent in Y. In these data the original standard deviation in Y is 5.1 per cent and the standard error of estimate ($s_{Y.X}$), which indicates the variation in Y after removing that associated with the X's, is 3.6 per cent.

Results of artificial rumen assays conducted on the 6 hays in Group II with *washed cell* inoculum are summarized in Table 4. The coefficients of correlation for per cent D.M. loss *in vitro* with per cent D.M. digestibility

TABLE 4.—GROUP II HAYS—CRUDE PROTEIN CONTENTS, DRY MATTER LOSSES *in vitro* AND DRY MATTER DIGESTIBILITY COEFFICIENTS AS DETERMINED ON THE FRESH FORAGES WITH SHEEP

Hay	Crude protein %	Dry matter loss, % (<i>washed cell</i> inoculum)		Dry matter digestibility coefficient <i>in vivo</i> Y
		X ₁ 24 hours	X ₂ 48 hours	
Mixture ¹	20.1	37	57	77
Mixture ¹	19.4	38	56	72 ²
Mixture ¹	15.9	32	50	62 ²
Alfalfa-timothy	20.9	39	53	74
Alfalfa-timothy	18.4	35	52	67
Brome grass	13.3	31	51	58

¹ Mixture of timothy, brome grass, alfalfa, red clover and Ladino clover which changed markedly in species composition at successive cuttings during the season, 1956.

² Estimated from fecal nitrogen.

in vivo are 0.94 and 0.82, and the regression coefficients 2.05 and 2.00, for the 24- and 48-hour incubation periods respectively. These r values are similar to those in Table 3 and significant at $P < 0.01$ and $P < 0.05$ respectively. However, the pooled regression coefficient, b_{YX1-2} , is 2.0, which indicates that for the hays and assays in Table 4 a change of 1 per cent in D.M. loss *in vitro* corresponded to a change of 2 per cent in D.M. digestibility *in vivo*, which is four times the comparable figure for data in Table 3. The difference between the regression coefficients for Group I and Group II hays is highly significant ($P < 0.01$).

GENERAL DISCUSSION

The results of these assays on limited numbers of samples from two groups of hays of separate origin and different quality, as indicated by proximate analyses and digestibility trials with sheep, offer some support for the premise that it may be possible to develop miniature artificial rumen assay methods for application in assessment of the nutritive value of hays and forages. Coefficients of correlation between both V.F.A. production and D.M. loss *in vitro* and D.M. digestibility *in vivo* were of the order of 0.7-0.8 and either significant or highly significant.

However, V.F.A. yields and D.M. losses were highly significantly correlated with the crude protein contents of the hays, and values for crude protein content were as highly correlated with D.M. digestibility ($r = 0.91$) as were the artificial rumen assay data. Further investigation is obviously required to appraise in what respects and to what extent, if any, assessments of the relative value of hays by artificial rumen assay may be superior or complementary to estimates based simply on relative protein levels. Assays on hays similar in protein content but differing in D.M. digestibility would be useful in this connection, as would the results of partial and multiple correlation analyses if sufficient data were available.

Problems awaiting solution in relation to the possible development of artificial rumen assay methods which would furnish data from which D.M. digestibility coefficients *in vivo* could be calculated in an absolute, as compared to a relative sense appear more complex. For example, no adequate explanation is apparent for the fact that from the data for hays in Group I of this study an increase of 1 per cent in D.M. loss *in vitro* corresponds to an increase of approximately 0.5 per cent in digestibility *in vivo*, whereas from the data for the hays in Group II the corresponding figure for increase in digestibility *in vivo* is 2 per cent.

ACKNOWLEDGEMENTS

The co-operation of J. G. O'Donoghue and other members of the staff of the Veterinary Laboratory, Alberta Department of Agriculture, Edmonton, in cannulating the sheep used in this study is gratefully acknowledged.

The digestion coefficients and crude protein values for the forages in Table 4 are unpublished data obtained by one of the authors (W.J.P.) in a co-operative project with G. J. Brisson, Chemistry Division, Science Service, Canada Department of Agriculture, Ottawa.

REFERENCES

1. Baumgardt, B. R., and D. L. Hill. Factors affecting the dry matter digestion of various roughages in the artificial rumen. *J. Dairy Sci.* 39: 943. (Abstr.) 1956.
2. Bell, J. M., G. H. Bowman, and R. T. Coupland. Chemical composition and digestibility of forage crops grown in central Saskatchewan, with observations on *Kochia* species. *Sci. Agr.* 32: 463-473. 1952.
3. Burroughs, W., N. A. Frank, P. Gerlaugh, and R. M. Bethke. Preliminary observations upon factors influencing cellulose digestion by rumen microorganisms. *J. Nutrition* 40: 9-24. 1950.
4. Cheng, E. W., G. Hall, and W. Burroughs. A method for the study of cellulose digestion by washed suspensions of rumen microorganisms. *J. Dairy Sci.* 38: 1225-1230. 1955.
5. Dougherty, R. W. Permanent stomach and intestinal fistulas in ruminants: Some modifications and simplifications. *Cornell Vet.* 45: 331-357. 1955.
6. Gray, F. V., A. F. Pilgrim and R. A. Weller. Fermentation in the rumen of the sheep. I. The production of volatile fatty acids and methane during the fermentation of wheaten hay and lucerne hay *in vitro* by microorganisms from the rumen. *J. Exptl. Biol.* 28: 74-82. 1951.
7. Huhtanen, C. N., R. K. Saunders, and L. S. Gall. Fiber digestion using the miniature artificial rumen. *J. Dairy Sci.* 37: 328-335. 1954.
8. Keeney, M. Direct chromatographic determination of C₁ to C₆ fatty acids in rumen fluid. *Univ. Maryland Agr. Expt. Sta. Misc. Publ.* 238, pp. 23-24. 1955.
9. Louw, J. G., H. H. Williams, and L. A. Maynard. A new method for the study *in vitro* of rumen digestion. *Science* 110: 478-480. 1949.
10. Martson, H. R. The fermentation of cellulose *in vitro* by organisms from the rumen of sheep. *Biochem. J.* 42: 564-574. 1948.
11. Phillipson, A. T., and J. R. M. Innes. Permanent stomach fistulae in ruminants. *Quart. J. Exptl. Physiol.* 29: 333-341. 1939.
12. Pigden, W. J. A microbiological method for evaluating forage quality. Ph.D. thesis, Univ. Saskatchewan, Saskatoon, Sask. 1955.
13. Pigden, W. J., and J. M. Bell. The artificial rumen as a procedure for evaluating forage quality. *J. Animal Sci.* 14: 1239. (Abstr.) 1955.

INTERPRETATION OF INTERACTION IN THE ANALYSIS OF VARIANCE OF A FACTORIAL EXPERIMENT

G. C. ASHTON, J. C. RENNIE and E. ETTER¹

*Departments of Physics and Animal Husbandry,
Ontario Agricultural College, Guelph, Ontario*

[Received for publication September 25, 1958]

ABSTRACT

Frequently imposed experimental treatments in biological research produce results which require evaluation in terms of interdependence between the factors under study. Data from a 2-factor calf-feeding experiment have been used to illustrate the inadequacy of over-all means in describing the treatment effects where factor interdependence existed. It was shown that, to make accurate appraisal of the treatment effects under these conditions, it is necessary to restrict consideration to the subtreatment means.

INTRODUCTION

Experiments in biology are characterized by response criteria that are invariably related to a complex of interrelated and interdependent processes. Measurement of the phenomenon of growth is a case in point. Hence, it is seldom to be expected that simple cause-result relationships will be exposed by imposed experimental treatments, but rather the results produced are likely to require evaluation in terms of interdependence. Such a situation suggests the use of the factorial experiment.

In a factorial experiment the effects of a number of different factors are studied simultaneously. The treatments tested then consist of all combinations that can be made up from the several factors under investigation.

The simplest case is a 2 x 2 factorial experiment which is characterized by two factors, each at two levels of intensity. This arrangement provides, in all, four treatment combinations. If A and B are used to symbolize the two factors and L and H to symbolize low and high levels of intensity, respectively, then the four combinations can be represented as AL, AH, BL and BH. This arrangement can be shown diagrammatically as

Factor A	
Factor B	BLAL
	BLAH
	BHAL
	BHAH

The factorial arrangement, by using all possible combinations of a number of factors, allows the average factor effects and, what is more important, their interdependence or interaction with one another to be estimated in the same experiment.

Interaction, therefore, may be thought of as the interdependence of one factor on another. That is to say, if the factors give responses that are independent of each other there is no interaction, or the results obtained

¹ Associate Professor of Statistics, Professor of Animal Husbandry, and Assistant in Animal Husbandry, respectively.

with one factor do not depend upon the presence or absence of the other. If the factors do not interact, then all of the observations on a treatment may be used to make main treatment comparisons. If, however, the factors do interact, then attention may have to be restricted to particular sub-treatments.

Frequently the objection is made that, where interaction is present, the possible restriction on the number of observations to be considered makes the factorial experiment an inefficient one. The fact is, however, that the factorial experiment is most worthwhile when the factors are dependent one on the other, i.e. interaction does exist. The existence of factor interaction can be verified only by employing a factorial arrangement.

EXAMPLE DATA

The data for this discussion were obtained from an experiment set up to compare four different kinds of milk diets for veal calves in the presence and absence of a dry supplement*. This was a 4 x 2 factorial experiment, with the one factor represented by four levels or kinds of milk namely, whole milk, WM, skim milk, SM, milk replacer, MR, and a nurse cow, NC; and the second factor represented by supplementation namely, no supplementation, Nil, and supplementation, Supp, of the several milk diets.

TABLE 1.—EXPERIMENTAL PLAN WITH DATA FOR DAILY GAIN, 1b.

Item	Nil	Supp.	Av.
WM	2.02	2.01	2.02
SM	1.54	1.93	1.74
MR	1.18	1.53	1.36
NC	2.59	2.29	2.44
Av.	1.83	1.94	1.89

Table 1 presents the pertinent information relating to the experimental arrangement. Thus there were four diets without supplement, which are indicated in the second column of the table, and four diets plus supplement, which are indicated in the third column of the table. To each of these eight sub-treatments four calves were allotted, each one of which constituted an experimental unit.

The data in this table are the daily gains per calf for the period from 3 days of age to approximately 200 lb. liveweight.

Table 2 presents the mean squares for an orthogonal biologically meaningful partition of the over-all treatment variability in the daily gains. This variability has been partitioned into seven fractions, four main comparison effects and three interaction comparison effects.

Orthogonal means that all seven treatment comparisons are made independent of each other. Biologically it seemed reasonable to compare NC against WM + SM + MR on the basis of a natural source of

* The dry supplement included hay and a calf meal, both fed *ad libitum*.

TABLE 2.—PARTITION OF THE OVER-ALL TREATMENT MEAN SQUARES FOR DAILY GAIN INTO COMPONENTS APPROPRIATE FOR SEVEN ORTHOGONAL TREATMENT COMPARISONS

Source of variation	Degrees of freedom	Mean squares
All treatment effects	7	0.829
Main effects	4	
Nil vs. Supp	1	0.0924
NC vs. WM + SM + MR	1	3.323 ¹
WM vs. SM + MR	1	1.175 ¹
SM vs. MR	1	0.574 ¹
Interaction effects (Supp x kinds of milk)	3	
Nil vs. Supp x NC vs. WM + SM + MR (A)	1	0.443 ¹
Nil vs. Supp x WM vs. SM + MR (B)	1	0.196 ¹
Nil vs. Supp x SM vs. MR (C)	1	0.0106
Experimental error	24	0.0370

¹ Mean squares are significant at $P = 0.05$.

milk versus man's manipulation of it; WM was compared against SM + MR on the basis of non-deficient versus possible deficient nutrients; and SM was compared against MR on the basis of non-substitution versus substitution. There appears to be no need for an explanation of the Nil against Supp comparison.

DISCUSSION

In the column of mean squares in Table 2 it is noted that the mean square for the over-all effect of Nil versus Supp is not significant. This is interpreted to mean that the average difference between non-supplemented and supplemented diets is no greater than might be expected from random variation. From this it would be concluded there is no real effect upon the response criterion from the supplementation of the diets, even though Nil had an average value of 1.83 and Supp had an average value of 1.93 lb. as indicated in Table 1.

The remaining three main comparisons have mean squares that are statistically significant. These are interpreted to mean that the differences measured in the several comparisons are greater than would be expected from random variation.

Before proceeding with a complete interpretation of these main effect mean squares, consider first the interaction mean squares. It is seen that two of the three interaction mean squares are significant. The interpretation of this situation is that the effects of the two factors, supplementation and kind of milk, are not independent of each other, but that the magnitude of the response is dependent upon the kind of milk involved and whether or not supplementation has occurred. The third interaction mean square is not significant, indicating that the two factors have operated independently of each other when only skim milk and milk replacer of the one factor were involved in the comparison.

TABLE 3.—TWO-WAY ARRANGEMENTS FOR ISOLATION OF INTERACTION EFFECTS FOR DAILY GAIN, LB.

Item	Nil	Supp
<i>Interaction A; significant</i>		
NC	2.59	2.29
WM + SM + MR	1.58	1.82
<i>Interaction B; significant</i>		
WM	2.02	2.01
SM + MR	1.36	1.73
<i>Interaction C; non-significant</i>		
SM	1.54	1.93
MR	1.18	1.53

In order to isolate the interaction effects, Table 3 was constructed.

Note, in Table 1, that the average response to the Nil group was 1.83 lb. and that for the Supp group was 1.93 lb., but that this observed superiority of the latter was not statistically significant. Calculations on the data in the upper section of Table 3 give an average response for the NC group of 2.44 lb. and for the WM + SM + MR group 1.70 lb. Note that the difference between these two groups was shown to be significant by the mean squares for this comparison in Table 2.

Consideration of the average sub-treatment responses show that, in the case of the NC treatment, the Nil group had a higher response than the Supp group which is an opposite effect to that observed, on the average, for Nil and Supp. In the case of WM + SM + MR group, the Nil treatment response was markedly less than that for the Supp group, 1.58 vs. 1.82 lb., respectively. Thus the greater value observed for the average response of Supp over that for the Nil group resulted entirely from the three groups other than the NC group. It is unrealistic, then, to state that supplementation of the milk diets had, on the average, effects upon the calf responses that were the result of sampling variation only, since in the NC group it gave a 0.30 lb. decrement while in the WM + SM + MR group it enhanced the response by 0.24 lb.

If the differences, Nil minus Supp, are extracted for the NC and WM + SM + MR groups, respectively, and in turn the difference of these differences, the value which the interaction A mean square of Table 2 measures is secured (1). For example:

$$\text{NC, } 2.59 - 2.29 = +0.30 \text{ lb.}$$

$$\text{WM + SM + MR, } 1.58 - 1.82 = -0.24 \text{ lb.}$$

$$\text{Difference} = +0.54 \text{ lb.}$$

From the fact that the mean square for this comparison is significant, it is concluded that this difference of +0.54 lb. is not the result of sampling variation only but that the factors supplementation and kinds of milk did not act independently of each other. In the light of this test, the first-mentioned conclusion derived from the over-all comparison, that the

apparent difference between Nil and Supp was the result of sampling variation, must now be rejected. Rather, the results to this point show that the effects of supplementation were to retard growth in the calves on the NC treatment but to enhance it in calves not on the NC regimen.

The average enhancement of 0.24 lb. ($1.82 - 1.58$) for Supp does not reflect the true picture for these three diets, however, since in the analysis of variance of Table 2 the mean square for interaction B, Nil vs. Supp \times WM vs. SM + MR, was significant. The interdependence of the two factors in this situation is revealed by the identical responses for Nil vs. Supp for the WM group, but a marked difference in response for the SM + MR groups. Instead of an indication that supplementation of the three milk diets enhanced the responses, the data show no effect of Supp for the WM group but a significant increase in the average for the SM + MR group. Calculation of the interaction difference in the manner used above for interaction A yields $+0.01$ minus -0.37 equals $+0.38$ lb.

The average increase of 0.37 lb. by supplementation of the SM + MR diets adequately describes this effect, since the interaction mean square for Nil vs. Supp \times SM vs. MR, interaction C, was not significant. The difference between the differences amounted to $+0.36$ minus $+0.40$ equals -0.04 lb. and may be considered due to sampling variation. Under this set of conditions supplementation was as effective in combination with SM as with MR, within the limits of sampling variation, and the response to one factor did not depend upon the presence or absence of the second factor. Thus, the effect of supplementation in the presence of SM and MR could be indicated by the average response taken over SM and MR for Nil and Supp with values of 1.36 and 1.73 lb., respectively. Similarly, SM and MR may be compared from values obtained by averaging both treatments over Nil and Supp which yield 1.74 and 1.36 lb., respectively.

In the presence of interactions, the responses to different combinations of factors, and consequently the conclusions to be drawn, are dependent upon the particular combinations involved in the comparison. Consequently, the existence of dependent relationships between the factors under study precludes the unqualified use of over-all averages of main effects to adequately describe treatment effects. In the absence of interdependence between factors, the over-all means of main comparisons do adequately describe the true responses and may be used for the summarization of the results.

A summary of responses under the conditions of this experiment can be made up then as follows:

Item	Nil	Supp	Av.
NC	2.59	2.29	—
WM	2.02	2.01	—
SM	—	—	1.74
MR	—	—	1.36
Av. (SM + MR)	1.36	1.73	

SUMMARY

A set of experimental calf feeding data was taken and analysed by the method of analysis of variance to indicate the interpretation of the response data when interactions are present. The example experiment was a 4 x 2 factorial, made up of four kinds of milk diets as one factor, and supplementation and non-supplementation as the other factor.

It was found that, although means for the two supplementation treatments, taken over all four kinds of milk, were not different, supplementation did have significant effects on growth rate when tested with certain kinds of milk used. These effects proved to be of different magnitude for different kinds of milk diets. With one kind of milk, supplementation gave less gain than non-supplementation, while with another kind of milk the results were reversed.

It was also found that, where the means for the two supplementation treatments indicated no enhancement whatever of the response, certain kinds of milk showed a marked increase to supplementation.

Thus, in a factorial experiment, when the factors under study do not exert their influence independently (interaction present), adequate summarization cannot be made from the over-all means of the main effects. To make an accurate appraisal of the results under these conditions, it is necessary to restrict consideration to the subtreatment means.

REFERENCE

1. Snedecor, G. W. Statistical methods. Iowa Collegiate Press, Ames, Iowa. 1956.

INFLUENCE OF DIETARY FAT ON ENERGY CONSUMPTION AND DIGESTION AND ON NITROGEN UTILIZATION OF WEANLING RATS¹

J. P. BOWLAND², I. R. SIBBALD³, R. T. BERG² AND N. HUSSAR⁴

[Received for publication September 15, 1958]

ABSTRACT

Energy digestibility and nitrogen retention were determined with weanling albino rats of the Sprague-Dawley strain fed diets varying from 0 to 30 per cent corn oil and containing 3 ratios of Cal. apparent digestible energy per gm. apparent digestible nitrogen at each fat level. Vitamins, salts and pure linoleic acid were included in the diets at calculated constant levels relative to the apparent digestible energy content. A 7-day metabolism period followed a 7-day diet acclimatization period. Food consumption varied inversely with the digestible energy content of the diet so that intake of digestible Cal. remained essentially constant and gain in weight of the rats was similar at all fat levels. Nitrogen levels per se did not influence digestible energy consumption but an interaction existed between fat x nitrogen levels in the diet in relation to digestible energy intake. Efficiency of food utilization improved as either fat or nitrogen levels in the diet increased. Energy digestibility was decreased slightly by the addition of fat to a fat-free diet when allowance was made for the influence of non-nutritive cellulose levels in the diets. The addition of fat to the diet resulted in a significant improvement in nitrogen digestibility and gross nitrogen retention.

INTRODUCTION

The food intake of some species appears to be largely controlled by the available energy content of the diet. In the case of the weanling rat, a series of papers by Sibbald *et al.* (4, 5, 6, 7) reported a high inverse relationship between food intake and the ADE⁵ content of the diet but as all their purified diets contained 5 per cent corn oil (Mazola) as the sole source of fat no estimate of any extracaloric influence which fat might have had upon food intake was possible.

It is recognized that within limits the ratio of energy to nitrogen in the diet is probably of greater import than the absolute level of each, as demonstrated in the rat by Sibbald *et al.* (5). Controversy has arisen, however, regarding the influence of the nature of non-protein calorie sources on bodily functions, particularly on nitrogen retention. Thomson and Munro (10) stated that the main consequence of replacing the carbohydrate of a mixed diet by fat is a deterioration in the utilization of dietary protein, but that this change is transitory in nature. Other workers, for example Goettsch (2) and Calloway and Spector (1), found no difference in nitrogen retention between rats fed different fat levels indicating that fat merely substituted for carbohydrate as an energy source per se.

Under certain dietary regimes fat appears to have a greater nitrogen sparing effect than carbohydrate. When rats were subjected to caloric restriction nitrogen losses increased as the fat content decreased [Swanson (9)]. An increased efficiency of protein utilization by weanling rats was

¹ This study was supported in part by a grant from the National Research Council of Canada.

² Associate Professor, Department of Animal Science, University of Alberta, Edmonton, Alberta.

³ Present address: Department of Nutrition, Ontario Agricultural College, Guelph, Ontario; formerly Graduate Student, Department of Animal Science, University of Alberta.

⁴ Present address: Department of Animal Science, University of Alberta; formerly Graduate Student in the same Department.

⁵ The abbreviations ADE = apparent digestible energy, and ADN = apparent digestible nitrogen, will be used throughout the paper.

reported by Schreiber and Elvehjem (3) when fat replaced sucrose in sub-optimum protein diets; however, when the protein content of the diets was adequate, substitution of fat for sucrose above a level of 5 per cent led to a decrease in protein utilization.

In a recent paper Yoshida *et al.* (12) concluded from their studies that calorie and nitrogen utilization in the growing rat was not affected appreciably, although improved utilization was evident in some cases, by the fat content of the diet but that protein to calorie ratio is an important factor to be considered in interpreting such measurements.

The purpose of the present study was to determine whether fat fed at levels of from 0 to 30 per cent of the diet exerted any extracaloric influence upon food intake, energy digestibility and nitrogen utilization of weanling rats receiving several ratios of nitrogen to energy.

METHODS

Two male and two female weanling albino rats of the Sprague-Dawley strain were allotted to each of the 15 diets listed in Table 1. Fat supplied from a minimum of zero percentage of total dietary calories in ration series 1 and 1₁ to over 50 per cent of total calories in ration series 4. On a digestible calorie basis fat supplied approximately 70 per cent of total calories in ration series 4. A non-nutritive cellulose was included in all rations at a level of 30 per cent in order to ensure that even on the high fat diets an individual rat would void sufficient feces in a one-week metabolism period for chemical analyses.

It will be noted that the levels of the nitrogen source increase with the fat content of the diet; the purpose of this was to obtain three approximately standard ratios of Cal. ADE per gm. ADN at each fat level, rather than to use isocaloric diets. Two series of rats were placed on diets without fat. The energy to nitrogen levels in these two series were varied slightly in an attempt to cover the range of Cal. ADE per gm. ADN of the 10, 20 and 30 per cent fat level groups. As the fat content of the diets increased it was assumed that the ADE per 100 gm. of food would increase and consequently the food intake would decrease [Sibbald *et al.* (4, 5)]. In order to ensure that approximately the same levels of vitamins, salts and pure linoleic acid would be available to each animal, these factors were included in the diets at levels calculated to provide equivalent intakes at all levels of ADE.

Metabolism data were collected for an experimental period of 7 days, following a 7-day diet acclimatization period. The management of the experimental animals and the collection and analyses of feces and urine together with the methods of determining the ADE and ADN consumption and nitrogen retention were as detailed by Sibbald *et al.* (4).

A factorial experimental design with three protein and five fat levels was used. In previous studies, involving similar data and using the same strain of rats, no sex effect was present and there was no evidence of a sex effect in the trial reported; therefore sex was not included in the statistical analysis. F values from analyses of variance, as well as error mean squares representing 45 degrees of freedom, are included in Table 2. The error mean square was used as the denominator for all F values reported.

TABLE 1.—DIETS FED DURING ACCLIMATIZATION AND METABOLISM PERIODS¹

	Mazola oil	Nitrogen ² source "A"	Sucrose	Salts ³	Vitamin ³ mix	Linoleic ⁴ acid	Analysis		ADE/100 gm. food	ADE/ gm. ADN in food
							Gross energy	Gross nitrogen		
	%	%	%	%	%	gm.	Cal./100 gm.	mg./100 gm.	Cal.	Cal.
Diet										
1a	0	6.1	58.9	4.0	1.0	0.10	378	869	269	382
1b	0	8.5	56.5	4.0	1.0	0.10	385	1194	275	268
1c	0	13.6	51.4	4.0	1.0	0.10	395	1822	278	181
Mean							386	1295	274	277
1a	0	6.7	58.3	4.0	1.0	0.10	385	937	269	356
1b	0	9.3	55.7	4.0	1.0	0.10	392	1326	273	239
1c	0	15.0	50.0	4.0	1.0	0.10	392	2047	269	158
Mean							390	1437	271	251
2a	10	7.3	46.8	4.7	1.2	0.12	435	1024	306	362
2b	10	10.0	44.1	4.7	1.2	0.12	441	1398	312	262
2c	10	16.2	37.9	4.7	1.2	0.12	450	2282	314	157
Mean							442	1568	311	260
3a	20	8.5	34.6	5.5	1.4	0.14	483	1236	351	335
3b	20	11.6	31.5	5.5	1.4	0.14	493	1646	359	253
3c	20	18.8	24.3	5.5	1.4	0.14	501	2626	373	161
Mean							492	1836	361	250
4a	30	9.7	22.4	6.3	1.6	0.16	550	1429	414	347
4b	30	13.3	18.8	6.3	1.6	0.16	544	1876	408	251
4c	30	21.4	10.7	6.3	1.6	0.16	551	3032	416	155
Mean							548	2112	413	251

¹ All rations contained 30% Alphacel, a non-nutritive cellulose prepared by Nutritional Biochemical Corp., Cleveland, O.² A casein-lactalbumen mixture supplemented with amino acids (Sibbald *et al.*, 4)³ Sibbald *et al.*, (4)⁴ Included as a 25% alcoholic solution

TABLE 2.—SUMMARY OF RESULTS OF ADDING DIETARY FAT TO RAT DIETS OF VARYING ENERGY-NITROGEN RATIOS

Diet ¹	Fat level	Av. gain in wt.		Av. body wt. met. period	Per 100 gm. body weight ²					Food/ gm. gain	Digestibility		Retention		Est. protein gain ³
		Acclim. period	Metab. period		Av. weekly gain	Food cons.	ADE cons.	Gross N cons.	Dry matter		Energy	Nitrogen	Gross N	ADN	
	%	gm.	gm.	gm.	gm.	Cal.	mg.	%	%	%	%	%	%	%	%
1a	0	14	14	62	23	108	291	942	7.7	72	71	81	64	79	17
1b	0	22	23	75	30	112	309	1342	4.9	72	71	86	70	81	20
1c	0	26	26	80	32	114	315	2068	4.4	70	70	85	50	60	21
Mean	0	20	21	72	28	111	305	1451	5.3	72	71	84	62	73	19
1a	0	8	12	57	21	105	283	982	6.6	70	70	81	54	66	17
1b	0	25	31	83	37	128	350	1696	3.6	70	70	86	70	81	21
1c	0	35	31	88	36	112	300	2285	3.2	68	69	83	56	67	22
Mean	0	23	24	76	31	115	311	1655	4.5	69	70	83	60	71	20
2a	10	21	17	71	24	102	312	1048	6.0	69	70	83	69	84	19
2b	10	25	26	79	33	98	307	1366	3.8	68	71	85	71	83	19
2c	10	34	35	93	36	98	310	2231	2.8	67	70	88	57	65	22
Mean	10	27	26	81	31	99	310	1548	3.8	68	70	85	66	77	20
3a	20	20	21	71	29	97	340	1196	4.6	67	73	85	73	87	18
3b	20	33	32	91	35	89	319	1460	2.8	67	73	86	73	85	19
3c	20	33	35	92	39	86	319	2245	2.6	68	74	88	58	65	21
Mean	20	29	29	84	34	91	326	1634	3.1	67	73	86	68	79	19
4a	30	19	20	70	29	84	347	1194	4.2	66	75	84	69	83	18
4b	30	30	31	88	34	79	324	1485	2.5	66	75	87	73	84	20
4c	30	33	35	91	37	77	320	2327	2.2	66	75	88	59	66	22
Mean	30	27	29	83	34	80	330	1669	2.8	66	75	86	67	78	20
F values ⁴															
Fat level		1.7	3.2*	2.6	1.7	29.9**	1.9	6.0**	7.5**	33.7**	46.3**	4.0**	2.8*	1.7	0.2
Protein level		15.5**	30.6**	23.6**	15.4**	1.1	0.6	402	12.5**	5.2**	0.1	18.1**	22.7**	26.5**	6.9**
F x P		0.6	0.6	0.6	0.5	2.1	2.2*	2.1	1.2	0.8	1.4	1.4	1.1	1.2	0.2
Error square		82.7	42.8	118	44.1	83.9	757	17,271	1.22	1.44	1.44	5.52	54.6	73.1	10.5

¹ Diet *a* is low nitrogen; diet *b* is medium nitrogen, and diet *c* is high nitrogen in all cases.² Nitrogen retained x 6.25 expressed as a percentage of gain*—Significant at *P* < 0.05**—Significant at *P* < 0.01

RESULTS AND DISCUSSION

The results of the experiment along with significance levels from an analysis of variance of the data are summarized in Table 2.

Rate of Gain, Food Consumption and Efficiency of Food Utilization

During the 7-day acclimatization period the level of fat in the diet (energy level of the diet) had no significant influence on gain whereas rats receiving low nitrogen diets at all fat levels gained more slowly than those receiving higher levels of nitrogen. In the metabolism period, following acclimatization, nitrogen levels still exerted a highly significant influence on rate of gain while higher fat levels in the diet also resulted in increased rate of gain. When the rate of gain in the metabolism period was converted to a 100-gm. body weight basis, the fat or energy level no longer had a significant influence on gain. In this experiment rate of gain in weanling rats was influenced only to a limited extent by level of energy as supplied by fat, although there was a definite trend toward increased gain as energy in the diet increased. An increasing ratio of nitrogen to energy in the diet significantly improved rate of gain.

The above results on rate of gain are in general agreement with most reports with chickens, as for example Sunde (8), where higher energy diets containing fat did not increase rate of gain consistently. Food consumption per 100 gm. body weight by the weanling rats was closely related to the energy content of the diet, food intake decreasing as fat levels increased. This observation, which agrees with an earlier report of Sibbald *et al.* (5), indicates that rate of gain would not be expected to increase markedly with increased fat in the diet, unless fat exerted an extracaloric effect on gain, because food consumption decreased as energy in the diet increased and Cal. of digestible energy consumed remained essentially constant.

ADE consumption also tended to remain constant at varying nitrogen levels, but an interaction existed between fat x nitrogen levels. At 20 and 30 per cent levels of fat, ADE consumption was increased on low nitrogen diets in an apparent attempt by the rat to get additional nitrogen. Conversely, at 0 per cent fat level there was a decrease in ADE consumption on low nitrogen diets, although this was less pronounced than the reverse effect at high fat levels. Energy level in the diet, regardless of whether the source was carbohydrate or fat, was the major factor influencing level of food consumption by the rats in this experiment, but nitrogen level had an influence under certain conditions as outlined above. Sibbald *et al.* (7) noted a similar occurrence in rats fed protein sources of varying biological value.

Efficiency of food utilization improved as fat levels in the diet increased which is an expected occurrence. As nitrogen increased, regardless of energy level, food required per gm. gain decreased, indicating that, using food efficiency as the criterion of measurement, optimum ADN to ADE levels were not exceeded, and possibly not reached, in this study.

Digestibility of Dry Matter, Energy and Nitrogen

Dry matter digestibility decreased significantly as fat levels increased. Nitrogen levels also influenced dry matter digestibility with the lowest

Cal. ADE per gm. ADN resulting in a decreased digestibility at 0 or 10 per cent fat levels but with no influence of nitrogen at higher fat levels.

The percentage ADE was significantly increased at 20 and 30 per cent levels of fat as compared to 0 or 10 per cent fat levels. This observation is not, however, a true indication of what occurred. All diets contained 30 per cent non-nutritive cellulose which has been reported by Sibbald *et al.* (4) to be essentially non-digestible by the weanling rat. This cellulose has a gross energy of 420 Cal./100 gm. and therefore represented 32, 29, 26 and 23 per cent respectively of total calories in the 0, 10, 20 and 30 per cent fat diets. Assuming complete digestibility of energy sources other than cellulose, the theoretical percentages ADE in these diets would be, therefore, 68, 71, 74 and 77 per cent while in actual fact they were 70, 70, 73 and 75 per cent. On this basis, the data suggest that, if the corn oil had any influence on energy digestibility, it was to cause a decrease in the percentage ADE as the level of fat in the diet increased. Nitrogen levels in the diet had no effect on percentage ADE at any energy level.

Nitrogen digestibility increased progressively when 10 to 30 per cent fat was added to the diet as compared to a diet containing no fat. The percentage ADN also increased as nitrogen levels in the diet increased at all levels of fat studied, except with zero fat, where the optimum ADN appeared to be in the medium nitrogen to energy ratio.

Nitrogen Retention

Nitrogen retention as a percentage of gross nitrogen or digestible nitrogen varied as energy-nitrogen ratios in the diet varied. Nitrogen retention was low on high nitrogen rations, indicating inefficient use of nitrogen when an apparent excess was present. This observation is in contrast to the results for over-all efficiency of food utilization which was maximum at the highest nitrogen levels. Gross nitrogen retention also tended to be reduced on the no-fat diets at the low nitrogen level (rations 1a and 1j), probably indicating either a limitation of essential amino acids for high utilization and/or the use of body protein which would result in excretion of urinary nitrogen not of food origin. Metabolic fecal nitrogen excretion was not studied in this experiment, but it is possible that the increase in fibre intake as the percentage fat in the diets decreased might have increased M.F.N. and hence decreased apparent nitrogen digestibility. For example, Whiting and Bezeau (11) reported that increasing fibre in the ration increased M.F.N. and decreased protein digestibility in the pig.

Sibbald *et al.* (7) reported for the weanling rat that for maximum percentage of ADN retention the optimum level of ADE per gm. ADN for a number of nitrogen sources was 250 to 300 Calories. The results of this study support a calorie-nitrogen ratio in this area but suggest that, when nitrogen retention is used as a criterion of ration adequacy, not only must energy to nitrogen ratios be considered in diet formulation for weanling rats, but the lower and upper levels of both energy and nitrogen must be taken into account.

Gross nitrogen retention was influenced by the fat level in the diet, being higher for rations containing fat than for these containing no fat. On the basis of ADN retention fat levels had no significant influence, but there was a very definite trend toward higher retention in fat-containing diets.

An estimate of protein gain as a percentage of total gain indicated no influence from fat level but a definite increase in estimated protein gain in the rat as nitrogen levels were increased at all levels of fat in the diet.

These studies were conducted using corn oil as the source of fat and it is possible that other fat sources might give different results; therefore, the conclusions should be restricted to some extent.

In these studies fat in the diet resulted in an improvement in gross nitrogen retention but a probable decrease in energy digestibility. The improved nitrogen retention was evident at 10 to 30 per cent fat levels as compared to zero fat levels in the diet. As all rations had an excess of linoleic acid present, it does not seem that this improved retention can be attributed to the presence of the essential fatty acids. Digestibility of nitrogen was changed by varying fat levels and could partially account for the improved utilization of nitrogen in diets containing fat, but other influences following absorption appeared to play a part. These studies support the view that the addition of fat to a fat-free diet containing a source of essential fatty acids results in improved gross nitrogen retention that cannot be attributed to the caloric value of the fat.

ACKNOWLEDGEMENT

The authors are indebted to Hoffmann-La Roche, Inc., Nutley, N.J.; Fine Chemicals Division, American Cyanamid Ltd., Pearl River, N.Y.; Merck and Co., Ltd., Montreal, Que., and Charles Albert Smith, Toronto, Ont., for the vitamins used in this experiment.

REFERENCES

1. Calloway, D. H., and H. Spector. Nitrogen utilization during caloric restriction. I. The effect of dietary fat content. *J. Nutrition* 56: 533-544. 1955.
2. Goettsch, M. Minimum protein requirement of the adult rat for 28-day periods of maintenance of body weight. *J. Nutrition* 45: 609-620. 1951.
3. Schreiber, M., and C. A. Elvehjem. Water restriction in nutrition studies; level of fat and protein utilization. *J. Nutrition* 57: 133-145. 1955.
4. Sibbald, I. R., R. T. Berg, and J. P. Bowland. Digestible energy in relation to food intake and nitrogen retention in the weanling rat. *J. Nutrition* 59: 385-392. 1956.
5. Sibbald, I. R., J. P. Bowland, A. R. Robblee, and R. T. Berg. Apparent digestible energy and nitrogen in the food of the weanling rat. Influence on food consumption, nitrogen retention and carcass composition. *J. Nutrition* 61: 71-86. 1957.
6. Sibbald, I. R., J. P. Bowland, R. T. Berg, and A. R. Robblee. The food intake and nitrogen retention of weanling rats fed protein-free rations. *J. Nutrition* 62: 171-184. 1957.
7. Sibbald, I. R., J. P. Bowland, A. R. Robblee, and R. T. Berg. The influence of the nitrogen source on the food intake and nitrogen retention of weanling rats. *J. Nutrition* 62: 185-200. 1957.
8. Sunde, M. L. The effect of fats and fatty acids in chick rations. *Poultry Sci.* 35: 362-368. 1956.
9. Swanson, P. P. Influence of non-protein calories on protein metabolism. *Federation Proc.* 10: 660-669. 1951.
10. Thomson, W. S. F., and H. N. Munro. The relationship of carbohydrate metabolism to protein metabolism. IV. The effect of substituting fat for dietary carbohydrate. *J. Nutrition* 56: 139-150. 1955.
11. Whiting, F., and L. M. Bezeau. The metabolic fecal nitrogen excretion of the pig as influenced by the amount of fibre in the ration and by body weight. *Can. J. Animal Sci.* 37: 95-105. 1957.
12. Yoshida, A., A. E. Harper, and C. A. Elvehjem. Effects of protein per calorie ratio and dietary level of fat on calorie and protein utilization. *J. Nutrition* 63: 555-570. 1957.

ANNUAL CYCLES IN NUMBERS OF THE SHEEP KED, *MELOPHAGUS OVINUS* (L.)^{1,2}

W. A. NELSON³ and M. C. QUALLY⁴

Canada Department of Agriculture, Lethbridge, Alberta

[Received for publication September 17, 1958]

ABSTRACT

Sheep ked [*Melophagus ovinus* (L.)] populations were studied on barren and pregnant ewes in 1953-54, and on rams, wethers, and pregnant, barren, and ovariectomized ewes continuously from 1954 to 1957. All sheep in the 1954-57 study were obtained as 5-months-old lambs. Number of keds on wethers and on barren and ovariectomized ewes rose consistently to peak levels in January-February and declined again to very low numbers in the summer months. This appeared to be the basic pattern. Keds on pregnant ewes that conceived in October-November continued to increase in numbers until parturition (March-April) but on ewes that conceived in December they reached peak levels at the usual time (January-February). Following parturition decline in numbers was sharp and very low levels of infestation were noted in the summer months. Rams maintained consistently higher numbers than all animals except pregnant ewes throughout most of the study. Ked numbers on all but pregnant ewes were smaller and tended to reach peak levels earlier in the second and third years of the animal's life than in the first year. It is postulated that some form of host resistance to ked infestation develops toward the end of the first year of life and that ked increases each autumn result from a partial breakdown of this resistance.

INTRODUCTION

Bequaert (1) has reviewed exhaustively the literature on the sheep ked, *Melophagus ovinus* (L.). That this insect is much less abundant on adult sheep during the summer than in winter has been attributed by various authors to shearing, to hot weather, and to transfer to lambs (1). In Australia, Graham and Taylor (4) noted that sheep that had been heavily infested in early spring supported few keds in the summer although they were unshorn. They attributed this to development by the sheep of resistance to the parasite. In Britain, Macleod (5) also noted a decline in numbers during early summer and suggested that this was due to a change in the microclimate within the fleece. None of these authors indicated at what time the decline commenced.

In South Africa, Bosman *et al.* (2) reported a decline in ked numbers during the winter (July, August, and September) and ascribed it to low air temperatures, together with the resulting longer incubation period of puparia and the dying-off of the older generations. In Wales, Evans (3) reported a decline beginning in April and May. This decline was attributed solely to transfer of keds from the older sheep to the lambs. In Wyoming, Pfadt *et al.* (8) showed a decline on feeder lambs starting in December or January, when the numbers on range flocks were continuing to rise. This decline was attributed to the better level of nutrition of the feeder animals.

At Lethbridge, Whiting *et al.* (9) showed declines in ked numbers on feeder lambs beginning in December, January, or February. These declines began consistently in three consecutive years during the winter

¹ Contribution No. 102, Science Service Laboratory, Lethbridge, Alta.

² Based on part of a thesis presented by the senior author to McGill University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

^{3,4} Associate Entomologist and Technician, respectively, Livestock Insect Section.

feeding periods and continued until, at normal shearing time, there were few keds left on the animals. Associated with the declines were pronounced increases in numbers of dead keds. During the course of the experiments reported by Whiting *et al.* (9) and Nelson *et al.* (7) it was noted that ked numbers on pregnant ewes continued to increase after those on barren ewes and wethers had begun to decline. In addition, sheep breeders have suggested that perhaps rams carry more keds than other sheep.

With the above background of observations, it was felt that a study of the fluctuations in numbers of keds on sheep in various physiological states would prove fruitful. This is a report on a 4-year study of the changes that were observed in ked populations on pregnant, barren, and ovariectomized ewes and on rams and wethers.

METHODS

1953-54 Experiment

The first experiment was conducted during the winter of 1953-54, using 32 yearling Canadian Corriedale ewes. They were fed in two groups of 16, from October 22 to December 22, on alfalfa hay *ad libitum* and one-half pound of grain per head. One group was bred during the period October 8-November 8. On December 22 each group was randomly divided into two pens and one section of each was placed on alfalfa hay, grain, and salt, and the other on peavine silage, grain, and salt. Following parturition, pregnant groups were returned to alfalfa hay and grain.

All ewes were infested by hand on two occasions, in mid-October (50 female and 20 male keds per animal), and in late November (25 females and 10 males per animal), with keds that were collected the same day from naturally infested sheep near Lethbridge. Keds were counted at monthly intervals, starting on November 16, prior to the second applied infestation. Counts were made on each ewe 2 days after parturition and, subsequently, at intervals of 2 weeks. In this experiment, ked counts of the entire sheep were used to assess ked numbers (7).

1954-57 Experiments

The sheep used in the second experiment were obtained as 5-months-old lambs in September, 1954, and were of Canadian Corriedale, Rambouillet, and Romnelet breeding. The experiment was designed to continue for 3 years. Groups of eight animals were selected in the following five categories: rams, wethers, barren ewes, ovariectomized ewes, and pregnant ewes (to be bred in season).

Sheep were group-fed on alfalfa or grass hay during most of the period. Pregnant animals were group-fed supplementary oat chop at one-half pound per head. Depending on pasture space available, they were run together occasionally on grass during the summer.

For the pregnant ewe group the same eight ewes were bred during the first 2 years. For the third year, groups were rearranged and the eight ewes that had remained barren for the first 2 years were bred. In 1954, mating took place during December 1 to 31; in 1955 during October 18 to November 7; and in 1956 during October 26 to November 16. Three

ewes in 1954, two in 1955, and one in 1956 did not conceive; the results, therefore, are based on five, six, and seven ewes, respectively.

All the lambs were found to be infested with keds when they arrived at the laboratory. The number per animal was adjusted to between 50 and 75 by manually transferring keds at random from heavily infested lambs to lightly infested ones. The keds were not artificially disturbed again during the 3 years, except at shearing. At shearing in June 1955 the shearers left a narrow strip unshorn under the neck, extending to the brisket. As each fleece was shorn it was stretched on a wool-sampling table and living keds were removed and returned to the unshorn strip on their host. This procedure allowed keds to survive on the animals for a longer period, although decline in numbers continued. Most of the keds remained on the neck and shoulders, and puparia were deposited only in the unshorn strip or in the longer fleece near it. As the fleece grew, keds and puparia were found on other parts of the animal. This procedure was not followed in 1956 and all keds that were removed with the fleece were discarded.

Ked counts were made at monthly intervals except during the first winter, when they were done every 2 or 3 weeks. On sheep in full fleece, keds were counted on one side of the animal only, from backbone to mid-belly, as outlined by Nelson *et al.* (7). On newly shorn animals the keds on the entire animal were tallied at each count for about 3 months after shearing and half of the numbers for each animal were recorded. During the first and third years dead keds were tallied and discarded at each count and mortality was recorded as a percentage of the total numbers for that date. July and August counts were not made in the second and third years.

RESULTS AND DISCUSSION

1953-54 Experiment

Figure 1 shows that the average numbers of keds on both barren and pregnant ewes increased at about the same rate until February, at which time those on the barren ewes began to decline. Keds on pregnant ewes

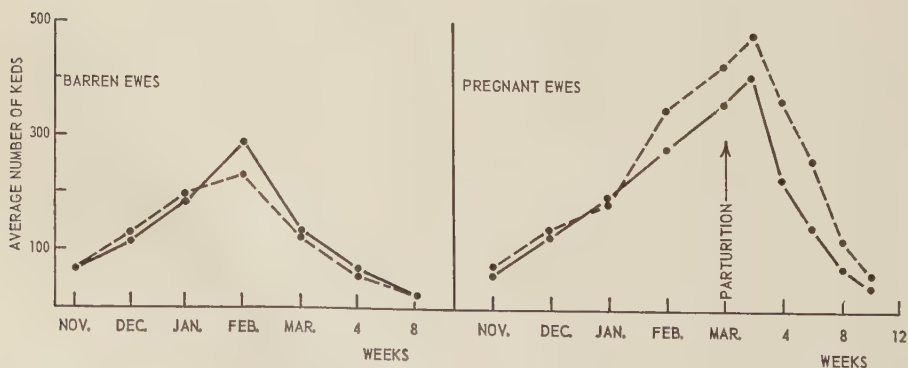


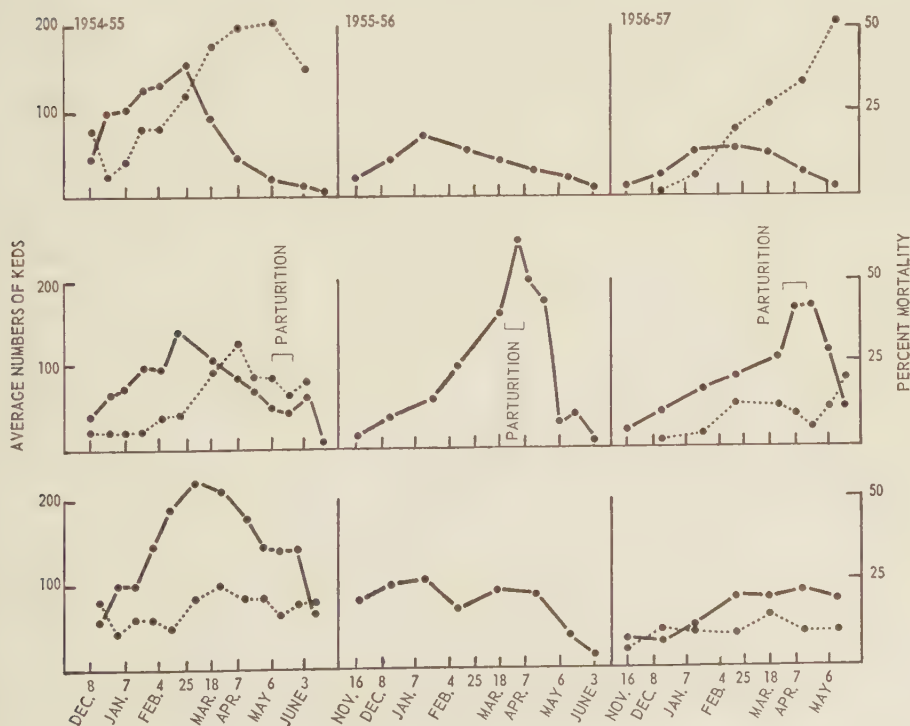
FIGURE 1. Average numbers of keds on eight barren and eight pregnant ewes fed alfalfa hay (solid line), and two similar groups fed peavine silage (dotted line).

continued their increase in numbers for another 6 weeks, but following parturition they also began to decline. Declines were accompanied by high ked mortality. There was considerable variation from sheep to sheep in the times that populations reached their peaks. In the sixteen barren ewes three peaks occurred in mid-December, five in mid-January, and eight in mid-February. One of the pregnant ewes died in March. Of the remaining fifteen ewes, one showed a peak in mid-January, two in mid-February, three in mid-March, and nine in late March and early April. Peak populations for barren ewes ranged from 61 to 659 keds and for pregnant ewes from 110 to 1348 keds.

1954-57 Experiment

All ked counts in this experiment, both in Table 1 and in Figures 2, 3, and 4, represent about one-half the keds present. Ked populations on barren ewes, wethers, and ovariectomized ewes behaved in essentially the same way as they had done on barren ewes in the 1953-54 test, increasing in numbers until January and February, then declining continuously until shearing time (Figure 2). Curves for wethers and ovariectomized ewes have therefore been omitted from this discussion.

Similarly, ked increases on pregnant ewes (Figure 3) were extended from 6 weeks to 2 months beyond the January-February peak period in the



FIGURES 2 (top), 3 (centre), and 4 (bottom). Average numbers of keds (solid line) and per cent ked mortalities (dotted line) on three groups of sheep during three years. Figure 2—barren ewes; Figure 3—pregnant ewes; Figure 4—rams.

TABLE 1.—RANGE AND AVERAGE OF MAXIMUM KED NUMBERS FOUND ON FIVE GROUPS OF EIGHT SHEEP IN 1954-1957

Year	Ewes			Wethers	Rams
	Pregnant	Barren	Ovariectomized		
1954-55	25-272 (145)	45-297 (151)	63-419 (210)	38-322 (201)	60-457 (256)
1955-56	52-539 (195)	18-138 (64)	16-245 (71)	28-114 (65)	28-254 (115)
1956-57	70-474 (183)	28-129 (57)	23-129 (61)	16-113 (66)	37-178 (109)

last 2 years. During the first year the peak period was reached in February and was followed by a slower decline than in other groups in the same year. The second peak at parturition was due to a great increase in keds (281) on one of the five ewes. Numbers on the other four ewes did not rise.

During the first year, rams were more susceptible to keds than were other groups. The cyclic rise and decline in numbers was noted on rams also (Figure 4) but numbers were maintained over longer periods compared with other groups. Likewise, mortality was maintained rather consistently below 25 per cent for several months. Certain individual rams maintained relatively static populations for periods up to 2 months, while others showed two or more peaks in ked numbers. While the residual summer ked population on sheep in all other groups averaged fewer than 5 keds per animal in the first year, that on rams averaged 15.

There appears to be little evidence that the decline in numbers of keds that starts in January or February each year is due directly to low air temperatures, as was postulated by Bosman *et al.* (2). If this were so, one would expect their numbers to increase again when warm spring weather appeared. Moreover, in another experiment at the Lethbridge Laboratory, in which six ewe lambs were kept at $50 \pm 5^\circ$ F. from December to April, the average numbers of keds were as follows: December 29, 96; January 27, 161; February 10, 211, February 24, 190; March 23, 99. Nor can the decline be attributed entirely to transfer of keds to lambs, as it has been shown that only newly emerged keds move from the ewes to their lambs (6).

A view that appears to be more tenable at the present time is that of Graham and Taylor (4), who suggested, without supporting data, that there was developed in sheep a resistance that caused decline in ked numbers. These are the only authors up to the present who have examined the same sheep for periods long enough to arrive at this conclusion. The behaviour of ked populations on barren and pregnant ewes in 1953-54 had suggested that the sexual physiology of the ewe might have affected the keds. Therefore, in order to test this possibility an ovariectomized group was added in the 1954-57 experiments. However, as exemplified by the behaviour of keds on barren ewes, ovariectomized ewes, and wethers in the present work, there occurs a basic cyclic pattern in which ked numbers increase to peak levels in January and February, decline steadily until June, and remain at low levels until autumn.

Two rather startling variations in the basic pattern support the theory of host resistance. First, ked populations were maintained on rams well into the spring season. The erratic behaviour of keds on some rams

might have been due to transfer of keds, because warm weather had intervened before their numbers had declined. Keds may transfer from animal to animal when an increase in ambient temperature causes them to migrate to the fleece surface (3).

Second, the basic pattern was disturbed by the state of pregnancy in ewes in both experiments. The only exception occurred in the first year of the 1954-57 experiment, when ewes conceived late (December). On all other pregnant ewes increases in ked numbers were prolonged from 6 weeks to 2 months beyond the January-February period.

The resistance theory is further supported by the observations of Pfadt *et al.* (8), who attributed early decline in ked numbers to better nutritional levels of sheep, and of Whiting *et al.* (9). This has also been borne out by preliminary work at Lethbridge.*

Maximum numbers of keds on sheep in all groups except the pregnant ewes were lower during the second and third years of the 1954-57 experiment (Table 1). This occurred in spite of the fact that in some groups the late-autumn infestations during the second and third years were equal to or greater than those of the first year. There was also a tendency for peak levels to be reached earlier in the second and third years. With this additional evidence it is tempting to postulate not only the development of host resistance to keds but also the partial breakdown of this resistance each autumn. This breakdown might be attributed to inherent cyclic changes in host physiology or to the effects of environment on the host. Studies of the effects of hormones on the basic ked population cycle are in progress and will be reported in due course.

ACKNOWLEDGEMENT

The authors are grateful to R. Connell, formerly Officer-in-Charge, Veterinary Research Station, Canada Department of Agriculture, Lethbridge, Alta., for performing ovariectomy.

REFERENCES

1. Bequaert, J. The Hippoboscidae or louse flies (Diptera) of mammals and birds. I. Structure, physiology and natural history. *Entomologica Americana* 32, 33:1-442. 1953.
2. Bosman, S. W., M. L. Botha, and D. J. Louw. Effect of the ked on Merino sheep. South Africa Dept. Agr. Sci. Bull. 281. 1950.
3. Evans, G. O. Studies on the bionomics of the sheep ked, *Melophagus ovinus* L., in west Wales. *Bull. Entomol. Research* 40:459-478. 1950.
4. Graham, N. P. H., and K. L. Taylor. Studies on some ectoparasites of sheep and their control. I. Observations on the bionomics of the sheep ked (*Melophagus ovinus*). Australia Council Sci. Ind. Research Pamphlet 108, p. 9-26. 1941.
5. Macleod, J. The distribution of dynamics of ked populations, *Melophagus ovinus* Linn. *Parasitology* 39:61-68. 1948.
6. Nelson, W. A. Transfer of sheep keds, *Melophagus ovinus* (L.), from ewes to their lambs. *Nature* 181:56. 1958.
7. Nelson, W. A., S. B. Slen, and E. C. Banky. Evaluation of methods of estimating populations of the sheep ked, *Melophagus ovinus* (L.) (Diptera: Hippoboscidae), on mature ewes and young lambs. *Can. J. Animal Sci.* 37:8-13. 1957.
8. Pfadt, R. E., L. H. Paules, and G. R. DeFoliart. Effect of the sheep ked on weight gains of feeder lambs. *J. Econ. Entomol.* 46:95-99. 1953.
9. Whiting, F., W. A. Nelson, S. B. Slen, and L. M. Bezeau. The effects of the sheep ked, *Melophagus ovinus* (L.), on feeder lambs. *Can. J. Agr. Sci.* 34:70-75. 1954.

* Nelson, W. A. *Unpublished*.

LEGUME-GRASS SILAGE AS A ROUGHAGE FOR THE PREGNANT EWE¹

G. M. CARMAN, W. J. PIDGEN
S. R. HASKELL² AND K. A. WINTER

Canada Department of Agriculture, Ottawa, Ontario

[Received for publication May 2, 1958]

ABSTRACT

Four rations with the roughage consisting of silage alone, two-thirds silage and one-third hay, one-third silage and two-thirds hay, and hay alone, were fed to pregnant ewes during the three successive winter periods, 1955, 1956, and 1957. All groups received 0.9 lb. grain per ewe per day during the last 6 weeks prior to lambing.

In 1957, when the dry matter content of the silage was only 21.4 per cent, the weight losses of the ewes were high and varied directly with the amount of silage fed. In 1955 and 1956, the weight changes were comparable to those in the hay-fed groups. Silage feeding had no effect on birth weights, 28-day weights, or weaning weights of the lambs, and ewe fleece weights were not affected.

At birth 75 per cent of the lambs from the silage lots graded good or better, while 97 per cent of those whose dams were fed hay alone fell in these categories. Seventy per cent of the lambs born to silage-fed ewes survived to weaning compared with eighty-eight per cent of the lambs from the hay-fed group. Some of the ewes on grass silage lacked sufficient milk to support their lambs for a period of two weeks after lambing. T.D.N. intakes of the ewes on the rations containing silage alone and two-thirds silage, one-third hay were below the levels recommended in recognized feeding standards.

INTRODUCTION AND LITERATURE REVIEW

With the expansion of grassland farming in Canada there has been a growing interest in silage made from grasses and legumes as a roughage for all classes of livestock. Its suitability for dairy (10) and beef cattle (15) has been well established, but this is not the case for sheep, especially for pregnant ewes.

At the University of Kentucky (1), ewes fed 12 pounds per day of first-cutting alfalfa silage containing just over 30 per cent dry matter made 45 per cent better gains and produced larger, stronger lambs than those fed 5 pounds of good alfalfa hay daily. In later work at the same station (2, 3, 4), a comparison was made of alfalfa silage, blue grass silage, corn silage, and alfalfa hay as roughages for wintering pregnant ewes. Each silage was fed to make up 50 per cent of the dry matter of the roughage portion of the ration, along with alfalfa hay. During the 3 years corn silage consistently ranked last, while the ranking of the other roughages varied from year to year. However, all the rations produced satisfactory results.

Bell (6) of Ohio studied the use of grass-legume silage as a winter roughage for the pregnant ewe. He found that grass silage, when of good quality, was palatable and usable in sheep rations and may even be used as the sole roughage in the winter ration of ewes or growing ewe lambs.

¹ Contribution from Animal and Poultry Science Division, Experimental Farms Service, Ottawa, Ont.

² Present address:—Trinity College, Toronto, Ont.

Cunningham and Watson (5), in a study of the palatability of grass silage found that the taste for it had to be acquired. Stewart (14) suggested that a period of 15 days is necessary for the animals to get accustomed to grass silage.

Welch *et al.* (16) reported that, when two lots of ewes were fed hay and hay plus silage, no appreciable differences were noted in the ewe weight changes during pregnancy and lactation, or in lamb birth weights, lamb gains to the end of the test, and ewe fleece weights. Garrigus (8) states that pregnant ewes receiving no pasture or other roughage will eat 8 to 12 pounds of grass silage daily but better results are obtained when 3 pounds of the silage are replaced by 1 pound of good grass or legume hay.

Jordan (9) conducted a trail to study the relative values of alfalfa as hay or silage when each was fed as the sole roughage to pregnant ewes. The hay-fed ewes made better weight gains, produced heavier fleeces, and had a better record of numbers of lambs born and weaned. At the University of Alberta (7) good quality alfalfa-timothy silage was compared with good quality alfalfa-timothy hay. Even when supplemented with oats the silage-fed ewes lost weight during the gestation period while the hay-fed ewes gained weight even without grain feeding. There was little or no difference between groups in mortality or vitality of the lambs at birth, but lambs from the silage-fed ewes weighed less at birth and at 30 and 60 days of age. Some of the silage-fed ewes lacked sufficient milk to nurse their lambs.

An experiment to compare the feeding of legume-grass silage alone, in combination with hay, and hay alone as roughages for pregnant ewes was conducted during the winters of 1955, 1956 and 1957 at the Central Experimental Farm, Ottawa, and is reported here.

PROCEDURE

Ewes of Western range breeding, and crossbred ewes from Western range ewes and various breeds of Down rams were utilized on this project. Rams were placed with the ewes during the first week of November each year and only ewes that were bred and did not return to service were included in the project. During the last week of December the ewes were allotted on a within breed, age, weight and service-sire basis to four lots of 16 each in 1955 and 1956, and 18 each in 1957.

The majority of the ewes lambled during the first 2 weeks in April which gave a feeding period on the experimental rations of 100 to 110 days including the preliminary period. Immediately after lambing each ewe was transferred to the standard post-lambing ration of hay and grain as used for the rest of the flock.

The experiment was conducted in the same barn each year and under as constant between-year care and circumstances as possible. The ewes were bedded regularly with shavings. Exercise yards were adjacent to each pen and the ewes given access to them at all times. Fresh, ice-free water, and a mixture of bone meal and iodized-cobaltized salt in equal amounts were available at all times.

Each lot was randomly assigned to one of the experimental rations and, after a 2-week preliminary adjustment, records as outlined below were kept.

Body weights of the ewes were obtained at the beginning and end of the preliminary period, at the end of successive 28-day periods (referred to as Periods 1 and 2) and within 24 hours *post partum*. The lambs were weighed and classified at birth, and weighed again at 28 days. The ewe weight changes and body weights of the lambs were subjected to an analysis of variance. Fleece weights were obtained for all ewes on the project.

The silage fed was a legume-grass mixture, being approximately 80 per cent legume in 1955, and decreasing to less than 50 per cent in 1956 and 1957. The hay was first-cut legume-grass mixture approximating the silage insofar as possible.

The roughages were fed to the different lots in the following proportions:—

Lot 1—Silage, full fed.

Lot 2—Two-thirds of silage consumed by lot 1, one-third of hay consumed by lot 4.

Lot 3—One-third of silage consumed by lot 1, two-thirds of hay consumed by lot 4.

Lot 4—Hay, full fed.

TABLE 1.—REPRODUCTIVE PERFORMANCE, AVERAGE BODY WEIGHTS AND WEIGHT CHANGES OF EWES FED SILAGE ONLY, HAY ONLY OR SILAGE-HAY COMBINATIONS, 1955-1956-1957

Lot Number	1	2	3	4
Treatment	All Silage	2/3 Silage 1/3 Hay	1/3 Silage 2/3 Hay	All hay
Total no. of Ewes	50	50	50	50
No. of ewes not in lamb	4	1	3	3
No. of ewes aborted or died before lambing	3	5	3	2
No. of ewes lambled	43	44	44	45
Lambing percentage**	149	152	139	140
*Weight at:—				
Start of preliminary period lb.	122.4	117.5	120.2	123.1
End of preliminary period lb.	114.1	113.7	116.7	122.9
End of period 1 lb.	126.5	128.1	127.9	132.8
End of period 2 lb.	126.1	125.6	128.3	133.0
<i>Post-partum</i> lb.	114.4	110.7	117.3	118.9
*Weight changes from start 1955 of preliminary period 1956 to immediately <i>post-partum</i> , lb.	-3.8 -1.6 -16.8	-2.3 -0.7 -16.6	+0.8 -0.7 -9.4	-4.4 -0.7 -5.9
Av.	-7.4	-6.5	-3.6	-3.7

* Includes only ewes that lambled

** Number of lambs born over ewes lambled

Daily roughage consumption records were kept, and the amounts for Lots 2 and 3 were adjusted daily as required by changes in the intakes of Lots 1 and 4. The daily ration was divided into two equal portions and fed in early morning and late afternoon. Whole oats and bran mixed in a ratio of 9:1 respectively were added to the ration at the rate of 0.9 pound/ewe/day approximately 6 weeks prior to lambing.

Samples of silage and hay for dry matter determination were taken twice weekly. The silage was placed in tightly closed polyethylene bags and frozen until dry matter determinations were made. Crude protein and crude fibre determinations were made on representative samples of the hay and silage for each year.

RESULTS AND DISCUSSION

Each year, during the preliminary period, the ewes on all the silage treatments lost weight. This was attributed to a reduction in their feed intake which presumably resulted in a reduction in fill as well as some body tissue loss. This loss was as high as 11 pounds for the all-silage treatment in 1955, while ewes receiving all hay in the same year showed little change in weight. As a result, while the lots were balanced as to weight in so far as was possible at the commencement of the preliminary period, at the end of this period the four lots were not as well balanced. For this reason the statistical analyses reported are limited to weight changes from the start of the preliminary period to immediately *post partum*. Table 2 shows that these changes were highly significant ($p > 0.01$) between treatments in 1957 but were non-significant in 1955 and 1956. Ewe weight losses were inversely proportional to silage quality. On the basis of protein and dry matter content (Table 3) silage quality would be rated as highest in 1956, lowest in 1957 and intermediate in 1955.

Average daily roughage and dry matter intakes of the various lots are presented in Table 3. The dry matter, T.D.N., and D.C.P. intakes are compared with N.R.C. requirements in Table 4.

Dry matter intakes were inversely proportional to the amount of silage in the ration and were highest for the silage-fed lots in 1956 when the dry matter content of the silage was the highest. When compared to the N.R.C. requirements for a pregnant ewe gaining one-quarter pound daily, the aver-

TABLE 2.—ANALYSIS OF VARIANCE OF EWE WEIGHT CHANGES
FROM START OF PRELIMINARY PERIOD
TO IMMEDIATE POST-PARTUM

Source of Variance	1955		1956		1957	
	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.
Total	50		56		64	
Treatments	3	64.1	3	3.6	3	485.0**
Error	47	55.0	53	61.0	61	69.5

** Significant ($p > 0.01$)

TABLE 3.—CHEMICAL COMPOSITION OF FEEDS AND AVERAGE DAILY FEED INTAKE DATA FOR EWES

*Chemical Composition, Percent
(Dry Matter Basis)*

Year	Hay		Silage			Concentrate	
	Crude protein	Crude fibre	Crude protein	Crude fibre	Dry matter	Crude protein	Crude fibre
1955	13.3	42.1	11.6	37.8	22.6	—	—
1956	6.6	34.6	12.0	36.8	25.7	—	—
1957	9.1	39.1	9.6	39.0	21.4	—	—
Aver.	9.7	38.6	11.1	37.9	23.2	*12.0	*10.6

*Average Feed Intake
(Lb. per ewe per day)*

Lot 1			Lot 2			Lot 3			Lot 4	
Year	Silage	D.M.**	Silage	Hay	D.M.**	Silage	Hay	D.M.**	Hay	D.M.**
1955	7.2	2.4	4.4	0.8	2.4	2.6	1.4	2.6	2.5	3.0
1956	9.4	2.7	7.0	1.2	3.1	4.2	2.3	3.6	3.6	3.7
1957	9.6	2.5	6.4	1.2	2.9	3.2	2.3	3.3	3.5	3.7
Aver.	8.7	2.5	5.9	1.1	2.8	3.3	2.0	3.2	3.2	3.5

* Estimated from tables of average analyses, F. B. Morrison, "Feeds and Feeding", 22nd ed.

** D. M. figures included grain mixture fed during last 6 weeks prior to lambing.

TABLE 4.—THREE-YEAR AVERAGE INTAKES OF DRY MATTER, T.D.N. AND D.C.P. BY PREGNANT EWES COMPARED WITH N.R.C. REQUIREMENTS

*(Lb. per ewe per day)**Start of test until 6 weeks before lambing*

Lot No.	Dry Matter		T.D.N.		D.C.P.	
	Actual	N.R.C.**	Estimated*	N.R.C.**	Estimated*	N.R.C.**
1	2.2	2.7	1.3	1.5	0.15	0.13
2	2.6	2.7	1.6	1.5	0.17	0.13
3	3.0	2.7	1.8	1.5	0.19	0.13
4	3.4	2.7	2.0	1.5	0.21	0.13

Last 6 weeks of gestation

1	3.0	4.0	1.9	2.3	0.23	0.19
2	3.2	4.0	2.0	2.3	0.24	0.19
3	3.4	4.0	2.2	2.3	0.24	0.19
4	3.7	4.0	2.3	2.3	0.27	0.19

* Estimated from chemical analyses of feedstuffs using tables of average digestibility, F. B. Morrison, "Feeds and Feeding", 22nd ed.

** Nutrient requirements of domestic animals. V. Nutrient requirements of sheep. Natl. Acad. Sci., Natl. Research Council Pub. 504. 1957.

age daily intake of dry matter and T.D.N. was Low in lots 1 and 2. Conversely D.C.P. intakes exceeded the N.R.C. requirements for all groups. It is believed that the low dry matter and T.D.N. intakes, of Lots 1 and 2 were the main reason for the relatively poor performance of both ewes and their lambs from these treatments.

Lamb data are shown in Table 5. While the data show a trend towards an increase in birth weight (when corrected for sex and birth mate by method of least squares) as the amount of silage in the ration decreased, this was not statistically significant. The number of lambs born full term did not appear to be affected by treatment. However, there was a greater number of stillborn lambs in Lot 1 than in the other lots. In the silage groups the average per cent mortality to 28 days was about one and one-half times that of the hay group.

The effect of feeding the silage on the birth grade of lambs is quite evident as illustrated in Table 5. The data show that the vigour of the lambs at birth was affected by the feeding of the silage, even when this was fed as only part of the total roughage. A relatively high percentage of the lambs from the silage-fed ewes needed prolonged assistance to suckle and additional time in the lambing pens. This loss of vigour was sufficiently great to be of practical significance to the sheep farmer.

TABLE 5.—PERFORMANCE OF LAMBS FROM EWES FED
SILAGE ONLY, HAY ONLY AND SILAGE-HAY COMBINATIONS,
1955-1956-1957

Lot Number		1	2	3	4
Treatment		All Silage	2/3 Silage 1/3 Hay	1/3 Silage 2/3 Hay	All Hay
Lambs born	No.	64	67	61	63
Lambs born alive	No.	56	65	58	61
Stillborn	No.	8	2	3	2
Lambs raised to 28 days	No.	50	52	45	54
Mortality to 28 days	No.	14	15	16	9
	%	21.9	22.4	26.2	14.3
Average birth weight of all lambs*	lb.	9.0	9.5	9.5	10.1
Average weight of lambs at 28 days*	lb.	23.3	23.8	24.0	24.7
Lambs weaned	No.	47	49	43	54
Av. weaning weight	lb.	66.3	67.4	67.8	64.9
Percentage weaned of lambs born	%	73	73	71	86
Lambs graded at birth:					
Very Good	No.	1	0	0	1
Good	No.	48	51	49	60
Fair	No.	6	11	4	0
Poor	No.	1	2	2	0
Weak	No.	0	1	4	0
Dead	No.	8	2	3	2
Lambs graded Very Good or Good	%	76.6	76.1	80.3	96.8

* Corrected for sex and birth mate

While no effect of rations on the number of lambs born was indicated there were a number of ewes on the silage rations that were unable to supply milk for their lambs immediately after lambing, while all ewes on the hay ration had adequate milk. Within 2 weeks of lambing and a return to the regular hay and grain ration the silage-fed ewes appeared to be providing sufficient milk.

Analyses of the 28-day weights, when corrected for sex and birth mate, showed no significant differences between lots but the average weights varied inversely with the amount of silage fed.

In 1955, when the legume content of the silage was highest and the intake of dry matter the lowest, the ewes on all three silage treatments appeared to have an unduly large number of everted uteri, abortions, stillbirths and deaths. In all years a moderate number of the ewes on silage needed some hormonal or other care to prevent complete eversion of the uterus both before and after parturition whereas none of those on the hay ration required such treatment. Other workers (13) have shown the estrogen content of alfalfa silage to be relatively high, but any estrogenic effect here would be confounded with the relatively low levels of nutrition in Lots 1 and 2. However, this was not the case in Lot 3.

In general appearance, both before and for some months after lambing, the effect of feeding grass silage was visible in that the silage-fed ewes presented an unthrifty dry appearance, with several showing soft cots at shearing. Some shedding of wool was noted, particularly in the all-silage lot, but the average fleece weights of the different lots was not affected. The ewes on all or part silage needed more care and assistance at lambing than the remainder of the flock.

Since average grass silage is adequate as the sole roughage for beef and dairy cattle (10) (15), it is of interest to speculate as to why the pregnant ewe is apparently unable to sustain herself satisfactorily under similar conditions. The comparative nutrient requirements of ewes and beef cows during the gestation period help to explain these species differences. First, a 120-pound mature ewe has a maintenance requirement for T.D.N., almost double that of a 1200-pound mature beef cow per 100 pounds live weight (12). Second, the ratio of weight of dam to weight of offspring is much different in the two species. The birth weights of lambs in this experiment were 9.0 and 14.2 per cent of their dams' weights for singles and twins respectively, whereas in the beef herd at the Central Experimental Farm the calves' birth weights averaged 6.6 per cent of their dams' over a 2-year period, less than half of that for twin lambs. Hence the pregnant ewe must eat much larger amounts of dry matter per unit body weight than the cow and it would appear essential that a bulky feed such as grass silage should be of high quality to minimize the amount of dry matter required. Of the silage fed in this experiment only the material available in 1956 can be considered as high quality. It is of interest that, while performance of the ewes and their lambs on the high silage treatments in 1955 and 1957 was generally unsatisfactory, relatively satisfactory results were obtained on these treatments in 1956.

ACKNOWLEDGEMENTS

The chemical analyses were performed by the Analytical Chemistry Unit, Science Service, Ottawa. The routine feeding, care, and management of the experimental animals were done by R. D. Holt and J. Arcand, of the Sheep Section staff.

REFERENCES CITED

1. Agricultural Experiment Station, University of Kentucky, Lexington, Ky. 53rd Annual Report. 1940.
2. Agricultural Experiment Station, University of Kentucky, Lexington, Ky. 56th Annual Report. 1943.
3. Agricultural Experiment Station, University of Kentucky, Lexington, Ky. 57th Annual Report. 1944.
4. Agricultural Experiment Station, University of Kentucky, Lexington, Ky. 58th Annual Report. 1945.
5. Cunningham, J. M. M., and S. J. Watson. Grass silage for sheep and fattening cattle. Transactions of the Royal Highland and Agriculture Society of Scotland LXIII (1):1-8. 1951.
6. Bell, D. S. Sheep. Legume-grass silage for roughage. Ohio Agricultural Experiment Station, Wooster, Ohio. Bull. 659. 1945.
7. Bowstead, J. E. Silage for pregnant ewes. Press Bulletin, University of Alberta XLII:3-4. Edmonton, Alta. 1957.
8. Garrigus, W. P. Grass silage. Extension Div., Coll. Agr., Univ. Kentucky, Lexington, Ky. Circ. 361. 1949.
9. Jordan, R. M. Hay or silage as the only roughage for pregnant ewes. S. Dakota Farm and Home Research VI:50. 1955.
10. Logan, V. S. Grass silage in dairy cattle rations. Can. Dep. Agr., Ottawa, Can. Publ. 929. 1955.
11. Nutrient requirements of domestic animals. V. Nutrient requirements of sheep. Natl. Acad. Sci., Natl. Research Council, Pub. 504. Washington, D. C. 1957.
12. Recommended nutrient allowances for domestic animals. IV. Recommended nutrient allowances for beef cattle. Natl. Research Council, Washington, D. C. 1950.
13. Pieterse, P. J. S., and F. N. Andrews. The estrogenic activity of legume, grass, and corn silage. J. Dairy Sci. 39:81-89. 1956.
14. Stewart, J. Grass silage for sheep. Agriculture LVI. (5). 1949.
15. Sylvestre, P. E., and E. Mercier. Feeding grass silage to beef cattle. Can. Dept. Agr. Ottawa, Can. Pub. 955. 1955.
16. Welch, J. A., E. A. Livesay, and W. R. Lewis. Grass and legume silage for sheep. West V. Univ. Agr. Expt. Sta., Morgantown, West Va. Bull. 363, Part 4, p. 4. 1954.

THE INFLUENCE OF PROTEIN AND ENERGY CONTENT OF THE RATION ON LAMB AND WOOL PRODUCTION OF RANGE EWES¹

F. WHITING AND S. B. SLEN

Canada Department of Agriculture, Lethbridge, Alberta

[Received for publication July 7, 1958]

ABSTRACT

Two feeding experiments were carried out with range ewes to determine the influence of the protein and energy content of the ration on lamb and wool production. When the energy content of the ration was increased approximately 10 per cent during early pregnancy [digestible crude protein (D.C.P.) intake was approximately 0.14 pound and digestible energy intake was approximately 3.2 therms daily on the basal ration], body weight gains and wool production of the ewes were increased ($P < 0.05$ and $P < 0.01$, respectively), but birth weights of the lambs in one experiment and 6-week weights of the lambs in both experiments were not affected significantly. However, when the energy content of the ration was increased during late pregnancy and early lactation (D.C.P. intake between 0.12 and 0.24 pound and digestible energy intake approximately 3.7 therms in Experiment 1, and 2.7 therms in Experiment 2, daily on the basal ration), body weight gains of the ewes were not affected, wool production was depressed (not significant in all cases), birth and 6-week weights of single lambs and birth weights of twin lambs in Experiment 1 were not affected. In Experiment 2, increasing the energy content of the ration reduced ($P < 0.05$) the birth weights of twin lambs.

When the protein content of the rations was increased (from 0.16 to 0.20 to 0.24, and from 0.12 to 0.14 to 0.16 pound D.C.P. intake daily in Experiments 1 and 2, respectively), there was no increase in body weights of the ewes or birth weights of single lambs, but there was an increase ($P < 0.05$) in the birth weights of twin lambs and an increase ($P < 0.01$) in wool production of the ewes. Increasing the protein content of the rations increased the 6-week weights of single and twin lambs in Experiments 1 and 2 ($P < 0.05$). The results of these experiments suggest that the minimum average protein requirement for pregnant and lactating ewes was approximately 0.10 and 0.11 pound D.C.P. per pound of T.D.N. intake for maximum lamb and wool production, respectively.

A nitrogen-balance and wool-growth study using non-pregnant, mature ewes showed that increasing the energy intake at low levels of protein intake caused a reduction in the apparent but not the "true" digestibility of the protein, no change in the biological value of the protein, and a reduction in weight of clean wool produced. At a higher level of protein intake, an increase in energy content of the ration resulted in no change in the apparent or true digestibility of protein but in an increase in wool production.

INTRODUCTION

Previous experiments at Lethbridge (13, 14, 15) have shown that a level of 7 per cent protein [(0.13 pound digestible crude protein (D.C.P.) daily)] in the ration fed to mature ewes during pregnancy and lactation was inadequate for normal lamb and wool production. However, levels of protein in the ration above 10 per cent (0.23 pound D.C.P. daily) resulted in no greater production than a 10 per cent level. Other experiments indicate similar results (7, 8, 17). In most of these experiments essentially isocaloric rations were used. Very little information is available on the influence of different energy levels in the ration upon protein requirements of the mature ewe.

¹ Contribution from the Division of Animal and Poultry Science, Experimental Farms Service, Canada Department of Agriculture, Ottawa, Ontario.

The results of experiments with cattle and sheep, using digestibility and nitrogen retention as criteria of protein utilization, have shown that the addition of energy (starch, cerelose, or corn oil) to low-protein rations reduced the digestibility of the protein (6, 11, 19) and in some cases reduced the retention of nitrogen (3), while in others, the retention of nitrogen (6, 9, 18) was increased. Fraser and Nichols (4, 5) found that the addition of 1 pound of starch to a maintenance ration for wethers increased the body gains and weight of clean wool produced.

To determine whether the energy content of the ration influenced the protein requirements of ewes for lamb and wool production, two experiments were conducted from 1954 to 1956. In these experiments, the ewes were fed low-protein rations at two levels of energy intake from approximately 2 months before breeding until 6 weeks before lambing. The ewes were then subdivided into six lots and fed three levels of protein at each of two levels of energy intake. Increased energy content of the ration was obtained by the addition of corn starch and corn oil.

A third experiment was carried out, using mature non-pregnant ewes in metabolism stalls to determine the influence of two levels of protein, each fed at two levels of energy intake, on the digestibility and biological value of the protein and on wool production.

PROCEDURE

Experiment 1

Ninety-six 2-year-old ewes of mainly Corriedale breeding were allotted at random to two equal lots (A and B), approximately 2 months before breeding started. These ewes had raised lambs previous to being put on the feeding experiment. The daily ration fed to Lot A was composed of: grass hay, 1.2; wheat straw, 0.8; dried molasses beet pulp, 0.3; barley, 0.45; beet molasses, 0.2; and mineral mix, 0.05 pound per head. Lot B was fed the same daily ration plus corn starch, 0.3; and corn oil, 0.1 pound per head. The mineral mix was composed of 100 pounds bonemeal, 100 pounds salt, 50 pounds ground limestone, 10 gm. MnSO_4 , 30 gm. FeSO_4 , 10 gm. CoSO_4 , and 10 gm. KI.

In these and the other rations used in these experiments, the grass hay was fed in the chopped form and the remaining ingredients were ground, mixed, and pelleted. The ewes were provided with 10,000 i.u. vitamin A each once weekly in the form of a dry stabilized vitamin A concentrate. They were fed in individual stalls twice daily. Each ewe within each lot was offered the same daily amount of feed. All feed refusals were weighed back and recorded. The rations containing corn starch and corn oil were less palatable to the sheep than those not containing these ingredients.

The D.C.P. and digestible energy (therms) intakes of the ewes when fed these rations are shown in Table 1. The D.C.P. intake of the ewes on both rations and the digestible energy intake of the ewes in Lot A were approximately the same as those recommended by Pope *et al.* (12). The digestible energy intake of the ewes in Lot B was approximately 10 per cent greater than that in Lot A.

Approximately 6 weeks before lambing, the 96 ewes were subdivided at random into six lots of 16 ewes with the restriction that 8 ewes in each lot come from Lot A and 8 from Lot B. They were fed rations containing three levels of protein each at two levels of energy. The daily rations were as follows:

Lot No.	1	2	3	4	5	6
	pounds					
Grass hay	1.2	1.2	1.2	1.2	1.2	1.2
Wheat straw	0.8	0.8	0.8	0.8	0.8	0.8
D.M.B.P. ¹	0.3	0.3	0.3	0.3	0.3	0.3
Beet molasses	0.2	0.2	0.2	0.2	0.2	0.2
Barley	0.55	0.55	0.35	0.35	0.15	0.15
Linseed oilmeal	0.10	0.10	0.30	0.30	0.50	0.50
Mineral mix	0.05	0.05	0.05	0.05	0.05	0.05
Corn starch	—	0.3	—	0.3	—	0.3
Corn oil	—	0.1	—	0.1	—	0.1

¹ Dried molasses beet pulp

The D.C.P. and digestible energy intakes of the ewes on the various rations are shown in Table 2. All ewes received less digestible energy than recommended by Pope *et al.* (12). The ewes in Lots 1 and 2 received less D.C.P. than recommended, those in Lots 3 and 4 received approximately the recommended amounts, and those in Lots 5 and 6 received more than the amounts recommended (12).

The ewes were weighed individually at 28-day intervals and within 12 hours after lambing. The lambs were weighed at birth and at 7-day intervals thereafter until 6 weeks old. Birth weights and 6-week weights of female lambs were adjusted to male weights by adding the following amounts to the female weights:

Single at birth.....	0.3 lb.
Twin at birth.....	0.5 lb.
Single at 6 weeks.....	0.6 lb.
Twin at 6 weeks.....	0.8 lb.

These adjustments were based on 5 years' lambing data at Lethbridge from ewes similar to those used in this experiment. Twin lambs raised as singles were not considered in the 6-week weights because of small numbers. All ewes that had twins and that had sufficient milk to raise them were allowed to do so.

Digestibility coefficients of the proximate principles and energy content of the rations fed to Lots 1, 2, 5, and 6 were determined with three ewes on each ration. The ewes used were from the lots being fed the ration.

A 2 x 2 cm. area was tattooed on the right shoulder of each ewe at the start of the feeding experiment. These areas were clipped at 60-day intervals and total weight of clean wool, and fibre diameters and lengths were determined for each sample.

During the period of late pregnancy and early lactation, only the wool data from ewes producing and raising single lambs were analysed as a previous study (16) had shown that type of birth and rearing (i.e. single or

twins), had a significant influence on wool production. The number of ewes that gave birth to and raised twin lambs was too small to be used as a base for valid conclusions.

Experiment 2

Ninety-six 3-year-old ewes of Corriedale breeding (most of the ewes were those used in Experiment 1) were allotted at random to two lots (A and B) approximately 2 months before breeding. These ewes had been on irrigated pasture for approximately 2 months before being allotted.

The daily rations offered to the ewes were the same as those fed during Experiment 1. The approximate daily D.C.P. and digestible energy intake for Lots A and B are shown in Table 1, and for Lots 1 to 6 in Table 3. The ewes in Lots A and B (before breeding and during early pregnancy) consumed approximately the same daily amounts of D.C.P. and digestible energy as those in Experiment 1. However, during the last 6 weeks of pregnancy and during early lactation, difficulty was experienced in getting the ewes to consume their daily allowance of feed, and especially those on the rations containing corn starch and corn oil (i.e. Lots 2, 4, and 6). This resulted in none of the ewes in the various lots consuming as much D.C.P. and energy as the ewes in the same lots did in Experiment 1.

The same experimental procedures were followed as in Experiment 1, and the same data were collected.

Experiment 3

Four rations (two levels of protein each at two levels of energy) were fed to each of six mature ewes in a nitrogen- and energy-balance experiment using a randomized block design. The daily rations were as follows:

Ration	I	II	III	IV
	pounds			
Grass hay	0.5	0.5	0.5	0.5
Wheat straw	0.9	0.9	0.9	0.9
Molasses	0.2	0.2	0.2	0.2
Mineral mix	0.05	0.05	0.05	0.05
Barley	0.15	0.15	—	—
Corn starch	0.20	0.40	—	0.20
Corn oil	—	0.10	—	0.10
Linseed oilmeal	—	—	0.35	0.35

The D.C.P. and digestible energy intakes of the ewes fed these rations are shown in Table 4. These amounts are less than recommended (12). However, previous experiments indicated that ewes confined to metabolism stalls would not consume the recommended amounts.

Each ration was fed for 35 days (25-day preliminary period and a 10-day collection period). Wool growth data were collected from four 2 x 2 cm. areas on the right shoulder of each ewe 5 days after the beginning and end of each ration period. The 5-day delay in taking wool samples after the ration was changed was to minimize carry-over effect.

RESULTS AND DISCUSSION

Since the ewes in Experiment 2 did not consume as much feed as those in Experiment 1, the two experiments were not considered as replicates, but were analysed and are reported as two separate experiments.

In the discussion which follows, it is assumed that the results obtained when extra energy, in the form of corn starch and corn oil, was added to the ration was due to energy *per se* and not to some peculiarities of corn starch and corn oil different from other sources of energy. However, this may not be an entirely valid assumption (6, 11, 18).

Effect of the Energy Content of the Rations Fed Prior to Six Weeks Before Lambing

Ewes fed additional energy from approximately 2 months before breeding until 6 weeks before lambing made greater gains ($P < 0.05$) in body

TABLE 1.—AVERAGE WEIGHTS AND FEED CONSUMPTION OF EWES, AVERAGE BIRTH AND 6-WEEK WEIGHTS OF THE LAMBS, AND WOOL PRODUCTION AS INFLUENCED BY THE ENERGY CONTENT OF THE RATION PRIOR TO 6 WEEKS BEFORE LAMBING (Experiments 1 and 2)

	(1954-55) Experiment 1		(1955-56) Experiment 2	
	Lot A	Lot B	Lot A	Lot B
Av. daily feed intake (lb.)	2.8	3.0	2.8	3.1
Av. daily protein intake (lb.)	0.19	0.20	0.21	0.21
Av. daily D.C.P. intake (lb.)	0.13	0.13	0.14	0.13
Av. daily T.D.N. intake (lb.)	1.6	1.8	1.5	1.8
Av. daily dig. energy intake (therms)	3.2	3.6	3.0	3.6
Av. daily D.C.P./lb. T.D.N.	0.08	0.07	0.09	0.07
No. ewes per lot	48	48	48	48
No. ewes that raised lambs	47 ¹	39 ²	43 ³	38 ⁴
Av. body weight of ewes (lb.)				
2 months before breeding	116	117	112	114
At breeding	122	127	114	126*
6 weeks before lambing	134	143*	131	151*
Immediately after lambing	134	139	123	136*
6 weeks after lambing	104	110	100	106
Av. birth weight of lambs (corrected to male weights) (lb.)				
Singles	10.9(39) ⁵	11.3(31)	10.6(39)	10.4(30)
Twins	8.4(16)	8.1(24)	8.0(16)	7.4(30)*
Av. 6-week weight of lambs (corrected to male weights) (lb.)				
Singles	33.7(38)	34.8(29)	28.4(31)	28.0(23)
Twins	23.8(14)	23.3(10)	18.1(6)	18.3(16)
Av. weight clean wool (mg.) ⁶	141	134	126	137**
Av. fibre length (mm.)	64	63	57	61**
Av. fibre thickness (μ)	21	21	24	26**

¹ One ewe dry

² Two ewes dry, three died, and four lost their lambs

³ One ewe dry, one ewe died, and three lost their lambs

⁴ Two ewes dry, three died, and five lost their lambs

⁵ Numbers in parentheses are the number of lambs making up the average shown

⁶ Average 60-day weights of wool from a 2 x 2 cm. area, (beginning of experiment to 6 weeks before lambing)

* Significantly different from Lot A ($P < 0.05$)

** Significantly different from Lot A ($P < 0.01$)

weight, produced more clean wool ($P < 0.01$) in Experiment 2 (not in Experiment 1), but the addition had no effect, with one exception, upon the birth or 6-week weight of the lambs (Table 1). This exception was that, in Experiment 2, the twin lambs from ewes fed additional energy were lighter ($P < 0.05$) at birth than twins from those fed less energy.

Effect of the Energy Content of the Rations Fed after Six Weeks Before Lambing

Feeding additional energy during the latter stages of pregnancy and during early lactation had no influence at any of the three levels of protein intake on body weight gains of the ewes or birth weights of single lambs (Tables 2 and 3). At the two lower levels of protein intake, feeding additional energy reduced ($P < 0.05$) birth weights of twin lambs in Experiment 2 but not in Experiment 1. In a similar study on fattening beef cattle, Matsushima (10) obtained the lowest gains on the low protein-high energy ration. In Experiment 1, at the highest level of protein intake, feeding additional energy increased ($P < 0.05$) the 6-week weight of the single lambs, but had no effect at the other levels of protein. On the contrary, in Experiment 2, feeding additional energy at the two highest levels of protein reduced ($P < 0.05$) the weights of single lambs, but had no effect on twin lambs. The reason for this difference is not known.

TABLE 2.—AVERAGE FEED CONSUMPTION AND WEIGHTS OF EWES, BIRTH AND 6-WEEK WEIGHTS OF THE LAMBS, AND WOOL PRODUCTION AS INFLUENCED BY THE PROTEIN AND ENERGY CONTENT OF THE RATIONS DURING THE LATTER STAGES OF PREGNANCY AND DURING LACTATION (Experiment 1)

Lot No.	1	2	3	4	5	6
Av. daily feed intake (lb.)	3.0	3.1	3.1	3.4	3.1	3.4
Av. daily protein intake (lb.)	0.26	0.26	0.32	0.33	0.38	0.38
Av. daily D.C.P. intake (lb.)	0.16	0.15	0.20	0.20	0.24	0.24
Av. daily T.D.N. intake (lb.)	1.8	1.9	1.8	2.1	1.9	2.1
Av. daily T.D.N. intake (lb.)	1.8	1.9	1.8	2.1	1.9	2.1
Av. daily digestible energy intake (therms)	3.6	3.8	3.7	4.2	3.8	4.2
Av. daily D.C.P./lb. T.D.N. (lb.)	0.09	0.08	0.11	0.10	0.13	0.11
No. ewes per lot	16	16	16	16	16	16
No. ewes that raised lambs	16	14 ¹	13 ²	13 ³	15 ⁴	15 ⁵
Av. body weight of ewes (lb.)						
6 weeks before lambing	139	137	138	138	136	140
Immediately after lambing	133	135	135	136	136	140
6 weeks after lambing	102	112*	106	107	101	114*
Av. birth weight of lambs (corrected to male weights) (lb.)						
Singles	11.3(13) ⁶	10.8(11)	10.9(12)	11.2(9)	10.5(13)	11.6(12)
Twins	7.0(6)	7.8(8)	9.1(6)	8.5(10)	8.8(4)	8.1(6)
Av. 6-week weight of lambs (corrected to male weights) (lb.)						
Singles	33.1(13)	31.2(9)	34.7(11)	33.7(9)	34.5(13)	37.4(11)*
Twins	22.8(4)	18.2(2)	20.9(4)	26.4(6)	23.4(4)	25.5(4)
Av. weight clean wool ⁷ (mg.)	79	72*	99	98	110	108
Av. fibre length (mm.)	18	16	19	19	18	18
Av. fibre thickness (μ)	18	18	19	20	20	20

¹ One ewe died and one lost its lamb

² One ewe dry, one died, and one lost its lamb

³ One ewe dry, one lambd prematurely, and one died

⁴ One ewe dry

⁵ One ewe lost its lamb

⁶ Numbers in parentheses are the number of lambs making up the average

⁷ Average 60-day weights of wool from a 2 x 2 cm. area

* Significantly different from those on the lower-energy ration but having the same protein content ($P < 0.05$)

TABLE 3.—AVERAGE FEED CONSUMPTION WEIGHTS OF EWES, BIRTH AND 6-WEEK WEIGHTS OF THE LAMBS, AND WOOL PRODUCTION AS INFLUENCED BY THE PROTEIN AND ENERGY CONTENT OF THE RATION DURING THE LATTER STAGES OF PREGNANCY AND DURING LACTATION (Experiment 2)

Lot No.	1	2	3	4	5	6
Av. daily feed intake (lb.)	2.6	2.6	2.6	2.6	2.6	2.8
Av. daily protein intake (lb.)	0.18	0.17	0.21	0.19	0.24	0.24
Av. daily D.C.P. intake (lb.)	0.12	0.11	0.14	0.12	0.16	0.16
Av. daily T.D.N. intake (lb.)	1.3	1.4	1.3	1.4	1.3	1.5
Av. daily digestible energy intake (therms)	2.7	2.8	2.7	2.8	2.7	3.2
Av. daily D.C.P./lb. T.D.N. (lb.)	0.09	0.08	0.11	0.09	0.12	0.11
No. ewes per lot	16	16	16	16	16	16
No. ewes that raised lambs	16	14 ¹	14 ²	10 ³	13 ⁴	14 ⁵
Av. body weight of ewes (lb.)						
6 weeks before lambing	141	140	142	140	143	137
Immediately after lambing	129	127	128	128	132	129
6 weeks after lambing	101	104	105	105	103	109
Av. birth weight of lambs (corrected to male weights) (lb.)						
Singles	10.6(12) ⁶	10.1(12)	10.3(10)	10.7(12)	11.1(10)	10.2(13)
Twins	7.6(8)	6.6*(8)	8.1(10)	6.6*(6)	8.1(10)	8.8(4)
Av. 6-week weight of lambs (corrected to male weights) (lb.)						
Singles	25.8(12)	26.8(10)	30.2(8)	26.2*(8)	30.6(9)	27.4*(12)
Twins	16.4(6)	17.0(4)	18.9(6)	—	20.9(4)	18.9(4)
Av. weight clean wool ⁷ (mg.)	55	47**	59	57	63	61
Av. fibre length (mm.)	15	15	15	15	18	16
Av. fibre thickness (μ)	17	17	18	17	20	19

¹ Two ewes died

² One ewe dry, and one lost its lamb

³ One ewe dry, one ewe died, and four lost their lambs

⁴ One ewe dry, two lost their lambs

⁵ One ewe died and one lost its lamb

⁶ Numbers in parentheses are the number of lambs making up the average

⁷ Average 60-day weights of wool from a 2 x 2 cm. area

* Significantly different from those on the lower-energy ration but having the same protein content ($P < 0.05$)

** Significantly different from those on the lower-energy ration but having the same protein content ($P < 0.01$)

On the lowest level of protein intake, feeding additional energy reduced wool production ($P < 0.05$ and $P < 0.01$ in Experiments 1 and 2, respectively), but had no influence at the higher level of protein intake.

Effect of Protein Content of Rations fed after Six Weeks Before Lambing

Increasing the levels of D.C.P. intake after 6 weeks before lambing had no effect upon body weight gains of the ewes, birth weights of single lambs, but increased ($P < 0.05$) the birth weight of twin lambs, 6-week weights of single and twin lambs, and wool production of the ewes (Tables 1 and 2). These results are in general agreement with those reported earlier (13, 14, 15).

On the basis of the results presented, the daily minimum requirement of D.C.P. of pregnant and lactating ewes was approximately 0.11 pound per pound of T.D.N. The results suggest, also, that the needs of the lambs are met before that for wool growth when the amount of protein supplied is below but close to the minimum requirements.

Nitrogen Balance Studies and Wool Growth (Experiment 3)

Data on the effect of level of protein and energy intake on the digestibility of protein, crude fibre, and energy; on the retention and B.V. of the protein; and on wool growth of non-pregnant ewes in digestion stalls are shown in Table 4. When the energy content of the low protein rations (I and II) was increased by the addition of corn starch and corn oil, there was a decrease ($P < 0.05$) in the digestibility of crude fibre, a decrease in the

apparent but not the "true" digestibility of protein, no change in the B.V. of the protein, and a decrease in the weight of clean wool produced. At the higher level of protein intake (III and IV), an increase in energy content of the ration resulted in no change in the apparent or true digestibility of protein or in the digestibility of energy, but a decrease ($P < 0.05$) in the digestibility of crude fibre and an increase in wool production. However, because ration IV supplied both more protein (although not formulated to do so) and more energy than Ration III, any differences between these two rations must be considered as being caused by a difference both in energy and protein. The lower amount of wool produced when the energy content of the low-protein rations was increased (reduced D.C.P. intake per pound T.D.N. from 0.07 to 0.05 pound) confirmed the results obtained in Experiments 1 and 2.

When the protein content of the rations was increased, there was an increase ($P < 0.05$) in the apparent digestibility of the protein, a reduction ($P < 0.05$) in the B.V. of the protein, and an increase ($P < 0.01$) in the weight of clean wool. The latter change occurred only on the higher level of energy intake. The reason for the lack of increase in wool production on the lower-energy rations when protein intake was increased was probably due to insufficient energy available for greater wool production.

TABLE 4.—THE EFFECT OF PROTEIN AND ENERGY INTAKE OF MATURE EWES ON THE DIGESTIBILITY AND BIOLOGICAL VALUE OF THE RATION AND WOOL GROWTH

Ration		I	II	III	IV
Av. weight of ewes	(lb.)	162	160	159	161
Av. daily feed intake	(lb.)	2.0	2.3	2.0	2.3
Av. daily protein intake	(lb.)	0.13	0.13	0.19	0.21
Av. daily energy intake	(therms)	3.5	4.1	3.5	4.1
Apparent digestibility of protein	(%)	51	46	63	63
True digestibility of protein ¹	(%)	91	91	87	90
Digestibility of crude fibre	(%)	39	33	45	33
Digestibility of energy	(%)	59	58	59	56
Av. daily D.C.P. intake	(lb.)	0.07	0.06	0.12	0.13
Av. daily T.D.N. intake	(lb.)	1.0	1.2	1.0	1.1
Av. daily D.C.P./lb. T.D.N.	(lb.)	0.07	0.05	0.12	0.12
Av. daily protein retained	(gm.)	6	4	17	17
B.V. of protein		80	81	69	68
Av. weight clean wool produced (35 days) (mg.)		69	55*	65**	79

¹ Metabolic fecal nitrogen assumed to be 0.5 gm. per 100 gm. dry matter intake

* Significantly lower than that obtained from ration I ($P < 0.05$)

** Significantly lower than that obtained from ration IV ($P < 0.01$)

The results from lamb and wool growth indicate, as has been previously reported (1, 2, 3, 6, 18, 19) that adding starch or high-energy feeds to a low-protein ration reduces the apparent digestion of the protein. At protein intakes above minimum requirements, this depression of protein utilization apparently does not take place. This indicates that the minimum protein requirements of a ruminant animal, for any function at a given body weight, is not a fixed quantity but depends on the energy content of the ration, and hence should be stated in relation to the energy intake and the form of energy in ration.

REFERENCES

1. Burroughs, Wise, Paul Gerlaugh, B. H. Edgington, and R. M. Bethke. Further observations on the effect of protein upon roughage digestion in cattle. *J. Animal Sci.* 8:9-18. 1949.
2. Burroughs, Wise, L. S. Gall, Paul Gerlaugh, and R. M. Bethke. The influence of casein upon roughage digestion in cattle with rumen bacteriological studies. *J. Animal Sci.* 9:214-220. 1950.
3. Fontenot, J. P., W. D. Gallup, and A. B. Nelson. Effect of added carbohydrate on the utilization by steers of nitrogen in wintering rations. *J. Animal Sci.* 14: 807-817. 1955.
4. Fraser, A. H. H., and J. E. Nichols. Wool growth in sheep as affected by the carbohydrate content of the diet. *Empire J. Exptl. Agr.* 2:9-19. 1934.
5. Fraser, A. H. H., and J. E. Nichols. Wool growth in sheep as affected by the carbohydrate content of the diet. *Empire J. Exptl. Agr.* 3:75-79. 1934.
6. Head, M. J. The effect of quality and quantity of carbohydrate and protein in the ration of sheep on digestibility of cellulose and other constituents of the ration with a note on the effects of adding vitamins of the B-complex on the digestibility and retention of the nutrients of a hay ration. *J. Agr. Sci.* 43:281-293. 1953.
7. Jordan, R. M. Rations for wintering breeding ewes. *S. Dakota Agr. Expt. Sta. Bull.* 339. 1950.
8. Klosterman, E. W., M. L. Buchanan, D. W. Bolin, and F. M. Bolin. Levels and sources of protein in rations for pregnant ewes. *J. Animal Sci.* 10:257-265. 1951.
9. Lofgreen, G. P., J. K. Loosli, and L. A. Maynard. The influence of energy intake on the nitrogen retention of growing calves. *J. Dairy Sci.* 34:911-915. 1951.
10. Matsushima, J., R. W. Rice, T. W. Dawe, and C. H. Adams. Protein energy ratios for fattening cattle. *J. Animal Sci.* 16:1053. (Abstr.). 1957.
11. Pfander, W. H., and I. S. Verma. Physical factors that influence the response of sheep to added corn oil. *J. Animal Sci.* 16:1087. (Abstr.). 1957.
12. Pope, A. L., C. W. Cook, W. E. Dinusson, U. S. Garrigus, and W. C. Weir. Nutrient requirements of domestic animals. V. Nutrient requirements of sheep. *Rev.* 1957. Publ. 504, National Research Council, Washington, D.C. 1957.
13. Slen, S. B., and F. Whiting. Wool production as affected by level of protein in the ration of the mature ewe. *J. Animal Sci.* 11:156-165. 1952.
14. Slen, S. B., and F. Whiting. Lamb production as affected by level of protein in the ration of the mature ewe. *J. Animal Sci.* 11:166-173. 1952.
15. Slen, S. B., and F. Whiting. Further observations on the effect of level of protein in the ration of the mature ewe on wool and lamb production. *Sci. Agr.* 32:375-379. 1952.
16. Slen, S. B., and F. Whiting. Wool growth in mature range ewes as affected by stage and type of pregnancy and type of rearing. *Can. J. Agr. Sci.* 36:8-13. 1956.
17. Van Horn, J. L., W. H. Burkitt, G. F. Payne, G. C. Hughes, and F. S. Willson. Nutritional requirements of ewes wintered under range conditions. I. Protein requirements. *Montana Agr. Expt. Sta. Circ.* 56. 1951.
18. Williams, V. J., M. C. Nottle, R. J. Moir, and E. J. Underwood. Ruminal flora studies in sheep. IV. The influence of varying dietary levels of protein and starch upon digestibility, nitrogen retention, and the free microorganisms of the rumen. *Australian J. Biol. Sci.* 6:142-151. 1953.
19. Woods, W. R., C. M. Thompson, and R. B. Grainger. The effects of varying levels of protein and cerelose on the utilization of mature timothy hay by sheep. *J. Animal Sci.* 15:1141-1146. 1956.